Chemical Composition and Antimicrobial Activity of *Populus nigra* Shoot Resin

Christian Paetz[a], Almuth Hammerbacher[a,b], Riya C. Menezes[a], Felix Feistel[a], Christiane Weigel[c], Kerstin Voigt[c], and Bernd Schneider[a,*]

[a]*Max Planck Institute for Chemical Ecology, Hans-Knoell-Strasse 8, 07745 Jena, Germany*

[b]*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa*

[c]*Leibniz Institute for Natural Product Research and Infection Biology, Adolf-Reichwein-Straße 23, 07745 Jena, Germany*

schneider@ice.mpg.de

The chemical composition of *Populus nigra* shoot resin has been investigated by chromatographic and spectroscopic methods. The analyses resulted in identification of 19 known compounds. The resin exhibited low activity against selected microorganisms.

Keywords: *Populus nigra*, Shoot resin, Phenyl-propanoids.

Plants of the genus *Populus* L. are known to produce resinous excretions from glands of leaves [1] or stipules [2]. In addition, extrafloral nectar can be released from leaf glands and has been shown to be protective against fungal infections [3]. Under greenhouse conditions the production of shoot resin in *P. nigra* L. (Salicaceae) can be observed (Fig. 1). The resin is released from glands situated on the adaxial site of stipules. The phenomenon of shoot resin release in *P. nigra* has not been described so far. After collection, the shoot resin was subjected to chromatographic separation. Resulting fractions were analyzed by means of mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.

P. nigra shoot resin appears as a pale yellow mixture. Prior to chromatographic separation, the water-soluble part of the resin was analyzed by NMR spectroscopy (see Supplementary data, figure 1). The ^{1}H NMR spectrum revealed sucrose as the only sugar species present, together with the major phenolic components **12** and **13**. Consequently, sucrose was removed by means of reversed phase solid phase extraction (RP-SPE), yielding six fractions which were subjected to high performance liquid chromatography-mass spectrometric (HPLC-MS) analysis. Based on HPLC-MS data, RP-SPE fractions 1 to 3 were selected for separation by high performance liquid chromatography-photodiode array detection-solid phase extraction (HPLC-PDA-SPE) (Supplementary data, Figure 2). Mass spectra of RP-SPE fractions 4 to 6 did not indicate useful additional compounds and therefore these fractions were discarded. The collected HPLC-PDA-SPE fractions were subsequently subjected to NMR and high resolution mass spectrometrIC (HR-MS) analysis for elucidation of the main constituents.

Analysis of RP-SPE fractions 1 to 3 resulted in identification of 19 compounds (Fig. 2). RP-SPE fraction 1: (*E*)-caffeic acid (**1**) [4], (*E*)-ferulic acid (**2**) [5], (*E*)-isoferulic acid acid (**3**) [5], (*Z*)-3,4-dimethoxy cinnamic acid (**4**) [6], and (*E*)-*p*-coumaric acid (**7**) [5]. RP-SPE fraction 2: (*E*)-3,4-dimethoxy cinnamic acid (**5**) [7], (*E*)-isoferulic acid methyl ester (**6**) [8], (*E*)-*p*-methoxy cinnamic acid (**8**) [9,10], (*E*)-3,4-dimethoxy cinnamic acid methyl ester (**9**) [11], (*Z*)-caffeic acid 3-methylbut-3-enyl ester (**10**) [12], (*Z*)-caffeic acid 3-methylbut-2-enyl ester (**11**), (*E*)-caffeic acid 3-methylbut-3-enyl ester (**12**) [13], and (*E*)-caffeic acid 3-methylbut-2-enyl ester (**13**) [13]. RP-SPE fraction 3: (*E*)-caffeic acid 2-phenylethyl ester (**14**) [14], (*E*)-isoferulic acid 3-methyl-3-butenyl ester (**15**) [12], (*E*)-isoferulic acid 3-methyl-but-2-enyl ester (**16**), (*E*)-isoferulic acid 2-methylbut-2-enyl ester (**17**), (*E*)-isoferulic acid 2-phenylethyl ester (**18**) [15], and (*E*)-caffeic acid (2*E*)-3,7-dimethylocta-2,6-dienyl ester (**19**) [14]. To our knowledge, NMR data of compounds **10**, **11**, **15**, **16** and **17** have not been reported (SciFinder® search March 2016). For analytical data, see below.

Figure 1: *Populus nigra* cutting with shoot resin emerging from stipules (arrows).

For determination of antimicrobial activity, an aqueous suspension of lyophilized shoot resin and a methanolic solution of resin were tested against the bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium vaccae*, and the fungi *Sporobolomyces salmonicolor*, *Candida albicans* and

Penicillium notatum. The tests showed low activity for the methanolic resin solution (Supplementary data, table 1). The water-suspended, lyophilized shoot resin showed no activity against the tested microorganisms.

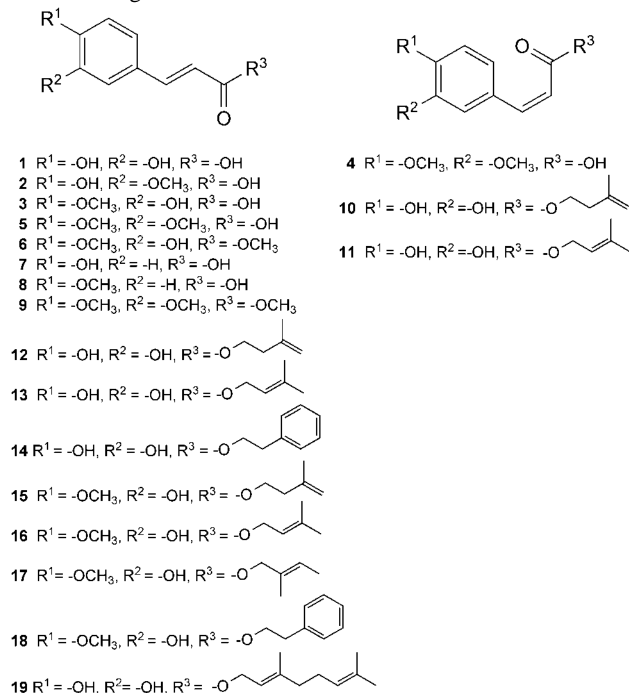

Figure 2: Chemical compounds identified in *Populus nigra* shoot resin.

The chemical profile determined for *P. nigra* shoot resin resembles results found for the analysis of propolis [16] with the difference being that in *P. nigra* shoot resin flavonoids have not been found. The main components **12** and **13** have been described previously in an analysis of *P. nigra* bud exudates [17] (i.e. washings of developing leaf buds). The occurrence of sucrose and phenylpropanoids, most of them esterified with prenyl or phenethyl moieties, in *P. nigra* resinous leaf excretions suggests a dual function of the shoot resin. The sucrose could play a role in rewarding ants or other insects for plant protective activities. Although to the best of our knowledge not reported for *P. nigra*, such symbiotic plant-insect interactions seem to be likely in the light of the present results and could be the subject of future ecological studies. The antimicrobial activity of the phenylpropanoids, although weak, could support the plant's defense against pathogenic microorganisms. In addition, or as an alternative function, the phenylpropanoids could be part of the reward to hypothetical symbionts in that these compounds might be sequestered into the insect's defence system.

Experimental

Plant material and sample collection: *P. nigra* shoot resin was collected in spring 2014 from plants emerged from cuttings. Greenhouse conditions were controlled keeping the temperature between 19–23°C (day) and 20–22°C (night). Relative humidity was between 50 % (minimum) and 60 % (maximum). The nectar was collected using pipettes with polypropylene tips.

Sample preparation: For chromatographic purification, 5 mL of nectar was diluted with 15 mL MeOH and kept refrigerated overnight to precipitate insoluble matter. After centrifugation (5 min at 16,100 rcf) the supernatant was passed through a HR-X SPE cartridge (500 mg) (Macherey-Nagel, Düren, Germany). Finally, the cartridge was washed with 4 mL of MeOH. The effluent was divided into 6 fractions which were subsequently evaporated by means of vacuum centrifugation. The fractions were reconstituted with 1 mL of MeOH and subjected to HPLC-MS analysis.

Analytical methods: For centrifugation, an Eppendorf 5804 R (Eppendorf AG, Hamburg, Germany) was used. Solvent evaporation was accomplished using a GeneVac HT-4X vacuum centrifuge (GeneVac Ltd., Ipswich, UK). LC-MS data were acquired using an Agilent 1100 HPLC system (degasser, quaternary gradient pump and column oven) (Agilent, Waldbronn, Germany) coupled to a J&M diode array detector (J&M Analytics AG, Essingen, Germany). Mass spectra were acquired in positive ionization mode using a Bruker Esquire 3000 mass spectrometer equipped with an ESI source (Bruker Daltonics, Bremen, Germany). Samples were measured in the positive ionization mode in the range *m/z* 150–1500 with skimmer voltage +33.9 V. Capillary exit voltage was +106.7 V, capillary voltage -2500 V, nebulizer pressure 35 psi, drying gas 12.0 l min^{-1}, and gas temperature 330°C. Fraction collection of selected peaks was accomplished using a Spark SPE system (Spark Holland B.V., Emmen, Netherlands) connected to the column outlet of the HPLC. HySphere resin GP cartridges were used. Using a make-up pump (WellChrom K-120, Knauer, Berlin, Germany), the effluent of the HPLC was enriched with a flow of 2.5 mL min^{-1} of water to achieve retention of the compounds on SPE. For control of the HPLC system and fraction collector Bruker Hystar ver. 3.2 was used. The mass spectrometer was controlled by Bruker Esquire control ver. 5.3. A Macherey-Nagel Nucleodur C18 Isis column (250 x 4.6 mm, 5 µm) (Macherey-Nagel, Düren, Germany) was used for chromatographic separation. Gradient elution conditions for HPLC-PDA-SPE were as follows: solvent A- water (0.1% formic acid), B- MeOH (0.1% formic acid). 0 min- 95% A, isocratic for 1 min, 60 min- 5% A, isocratic for 8 min, 69 min- 95% A, isocratic for 1 min. Prior to each injection, a 5 min equilibration period was set. Flow rate was 0.8 mL min^{-1}, column temperature was set to 35°C. NMR spectra were recorded on an Avance III HD 700 NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) operating at a resonance frequency of 700.13 MHz for ^{1}H and 175.75 MHz for ^{13}C. The spectrometer was equipped with a 1.7 mm Bruker TCI microcryoprobe. Standard Bruker pulse sequences were used to record spectra in MeOH-d_4 at 300 K. Spectra were referenced to tetramethylsilane, which was used as an internal standard. HR-MS data were recorded on a UHPLC Ultimate 3000 series RSLC (Dionex, Sunnyvale, CA, USA) connected to a LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). HPLC was performed using an Acclaim C18 column (150 × 2.1 mm, 2.2 µm, Dionex, Sunnyvale, CA, USA) with a constant flow rate of 300 µl min^{-1} of a binary solvent system of water (solvent A) and acetonitrile (solvent B) (hypergrade for LCMS, Merck, Darmstadt, Germany), both containing 0.1% (v/v) formic acid (FA; eluent additive for LC–MS, Sigma Aldrich, Steinheim, Germany). The following gradient was used: 0 min – 0.5 % B, 10 min - 10% B, 14 min – 80% B, 19 min – 80% B, 19.1 min - 0.5 % B, 25 min – 0.5 % B. Electrospray Ionization (ESI) source parameters were set to 4 kV for spray voltage, 35 V for transfer capillary voltage at a capillary temperature 275°C. The samples were measured in positive ion mode in the mass range of *m/z* 100 to 1500 using 30,000 $m/\Delta m$ resolving power in the Orbitrap mass analyzer. Data were interpreted using XCALIBUR software (Thermo Fisher Scientific, Waltham, MA, USA).

Antimicrobial activity: For determination of biological activity, two different samples of shoot resin, PnN1 and PnN2 were used (for details regarding bacterial and fungal strains as well as medium

compositions see Supplementary data, Table 2). PnN1 was a methanolic extract of shoot resin solubilized in 50% MeOH. PnN2 consisted of lyophilized shoot resin, re-suspended in water prior to use. Both samples were tested in a concentration of 10 mg mL^{-1}. Bioassays were conducted using bacteria and fungal cultures. *B. subtilis*, *S. aureus*, *E. coli, P. aeruginosa* and *M. vaccae* were cultivated on standard I nutrient agar (NA I) in Petri dishes. Bioassays were conducted at 37°C using bacterial and fungal cultures. *S. salmonicolor* and *P. notatum* were cultivated on malt agar (MA). *C. albicans* was cultivated on yeast morphology agar (YMA). Fungus assays were conducted at 30°C. After inoculation, a disc (9 mm in diameter) was removed from the center of the Petri dish and 50 µL of the test solution was added to the cavity. After 18 h of incubation at the respective temperatures the inhibiting areola was measured.

(*Z*)-Caffeic acid 3-methylbut-3-enyl ester (10)
UV/Vis (PDA, MeOH/H_2O) λ_{max} (nm): 219, 246, 317.
^{1}H NMR (700 MHz, MeOH-d_4): δ 7.30 (1H, d, J = 2.2 Hz, H_{ar}-2), 7.02 (1H, dd, J = 8.4/2.2 Hz, H_{ar}-6), 6.77 (1H, d, J = 12.3 Hz, H-3), 6.73 (1H, d, J = 8.4 Hz, H_{ar}-5), 5.72 (1H, d, J = 12.3 Hz, H-2), 4.78 (1H, s, =CH_{2a}), 4.72 (1H, s, =CH_{2b}), 4.24 (2H, dd, J = 7.1/7.1 Hz, CH_{2bu}-1), 2.36 (2H, dd, J = 7.1/7.1 Hz, CH_{2bu}-2), 1.75 (3H, s, -CH_3).
^{13}C NMR (175 MHz, MeOH-d_4): δ 168.5 (C-1), 147.9 (C-4_{ar}), 145.4 (C-3_{ar}), 143.0 (C-3_{bu}), 128.1 (C-1_{ar}), 124.6 (C-6_{ar}), 118.3 (C-2_{ar}), 116.6 (C-2), 115.6 (C-5_{ar}), 112.6 (C-4_{bu}), 61.8 (C-1_{bu}), 37.5 (C-2_{bu}), 22.4 (-CH_3).
HRMS-ESI: *m/z* [M + H^+] calcd for $C_{14}H_{17}O_4$: 249.1127; found: 249.1485.

(*Z*)-Caffeic acid 3-methylbut-2-enyl ester (11)
UV/Vis (PDA, MeOH/H_2O) λ_{max} (nm): 219, 246, 317.
^{1}H NMR (700 MHz, MeOH-d_4): δ 7.30 (1H, d, J = 2.2 Hz, H_{ar}-2), 7.02 (1H, dd, J = 8.4/2.2 Hz, H_{ar}-6), 6.77 (1H, d, J = 12.3 Hz, H-3), 6.73 (1H, d, J = 8.4 Hz, H_{ar}-5), 5.72 (1H, d, J = 12.3 Hz, H-2), 5.36 (1H, m, H_{bu}-2), 4.63 (2H, d, J = 7.2 Hz, H_{bu}-1), 1.76 (3H, s, -CH_3), 1.72 (3H, s, -CH_3).
^{13}C NMR (175 MHz, MeOH-d_4): δ 168.5 (C-1), 147.9 (C-4_{ar}), 145.4 (C-3_{ar}), 144.6 (C-3), 140.2 (C-3_{bu}), 128.1 (C-1_{ar}), 124.6 (C-6_{ar}), 119.6 (C-2_{bu}), 118.3 (C-2_{ar}), 116.6 (C-2), 115.6 (C-5_{ar}), 63.4 (C-1_{bu}), 25.7 (C-4_{bu}), 17.9 (-CH_3).
HRMS-ESI: *m/z* [M + H^+] calcd for $C_{14}H_{17}O_4$: 249.1127; found: 249.1121.

(*E*)-Isoferulic acid 3-methylbut-3-enyl ester (15)
UV/Vis (PDA, MeOH/H_2O) λ_{max} (nm): 214, 244, 297, 326.
^{1}H NMR (700 MHz, MeOH-d_4): δ 7.55 (1H, d, J = 16.0 Hz, H-3), 7.06 (1H, d, J = 2.0 Hz, H_{ar}-2), 7.04 (1H, dd, J = 8.2/2.0 Hz, H_{ar}-6), 6.94 (1H, d, J = 8.2 Hz, H_{ar}-5), 6.29 (1H, d, J = 16.0 Hz, H-2), 4.82 (1H, s, =CH_{2a}), 4.78 (1H, s, =CH_{2b}), 4.30 (2H, dd, J = 6.8/6.8 Hz, CH_{2bu}-1), 3.89 (3H, s, -OCH_3), 2.42 (2H, dd, J = 6.8/6.8 Hz, CH_{2bu}-2), 1.80 (3H, s, -CH_3).
^{13}C NMR (175 MHz MeOH-d_4): δ 169.0 (C-1), 151.8 (C-4_{ar}), 148.7 (C-3_{ar}), 146.3 (C-3), 143.1 (C-3_{bu}), 128.9 (C-1_{ar}), 122.4 (C-6_{ar}), 115.9 (C-2), 114.7 (C-2_{ar}), 112.6 (C-4_{bu}), 112.3 (C-5_{ar}), 63.5(C-1_{bu}), 56.2 (-OCH_3), 37.7 (C-2_{bu}), 22.4 (-CH_3).
HRMS-ESI: *m/z* [M + H^+] calcd for $C_{15}H_{19}O_4$: 263.1283; found: 263.1279.

(*E*)-Isoferulic acid 3-methylbut-2-enyl ester (16)
UV/Vis (PDA, MeOH/H_2O) λ_{max} (nm): 214, 244, 297, 326.
^{1}H NMR (700 MHz, MeOH-d_4): δ 7.56 (1H, d, J = 16.0 Hz, H-3), 7.07 (1H, d, J = 2.1 Hz, H_{ar}-2), 7.05 (1H, dd, J = 8.2/2.1 Hz, H_{ar}-6), 6.94 (1H, d, J = 8.2 Hz, H_{ar}-5), 6.32 (1H, d, J = 16.0 Hz, H-2), 5.41 (1H, m, H_{bu}-2), 4.68 (2H, d, J = 7.1 Hz, H_{bu}-1), 3.88 (3H, s, -OCH_3), 1.78 (3H, s, -CH_3), 1.76 (3H, s, -CH_3).
^{13}C NMR (175 MHz MeOH-d_4): δ 168.9 (C-1), 151.6 (C-4_{ar}), 148.4 (C-3_{ar}), 146.3 (C-3), 139.8 (C-3_{bu}), 128.9 (C-1_{ar}), 122.5 (C-6_{ar}), 119.9 (C-2), 116.0 (C-2_{bu}), 114.5 (C-2_{ar}), 112.3 (C-5_{ar}), 62.0 (C-1_{bu}), 56.2 (-OCH_3), 25.7 (C-4_{bu}), 17.9 (-CH_3).
HRMS-ESI: *m/z* [M + H^+] calcd for $C_{15}H_{19}O_4$: 263.1283; found: 263.2364.

(*E*)-Isoferulic acid 2-methylbut-2-enyl ester (17)
UV/Vis (PDA, MeOH/H_2O) λ_{max} (nm): 214, 244, 297, 326.
^{1}H NMR (700 MHz, MeOH-d_4): δ 7.56 (1H, d, J = 16.0 Hz, H-3), 7.07 (1H, d, J = 2.1 Hz, H_{ar}-2), 7.05 (1H, dd, J = 8.2/2.1 Hz, H_{ar}-6), 6.94 (1H, d, J = 8.2 Hz, H_{ar}-5), 6.32 (1H, d, J = 16.0 Hz, H-2), 5.60 (1H, m, H_{bu}-3), 4.56 (2H, s, H_{bu}-1), 3.88 (3H, s, -OCH_3), 1.70 (3H, s, -CH_3), 1.76 (3H, dd, , J = 6.8/1.0 Hz, H_{bu}-4, (-CH_3)).
^{13}C NMR (175 MHz MeOH-d_4): δ 168.9 (C-1), 151.6 (C-4_{ar}), 148.4 (C-3_{ar}), 146.3 (C-3), 132.2 (C-2_{bu}), 128.9 (C-1_{ar}), 124.6 (C-3_{bu}), 122.5 (C-6_{ar}), 119.9 (C-2), 114.5 (C-2_{ar}), 112.3 (C-5_{ar}), 70.9 (C-1_{bu}), 56.2 (-OCH_3), 13.5 (-CH_3). 13.2 (C-4_{bu}).
HRMS-ESI: *m/z* [M + H^+] calcd for $C_{15}H_{19}O_4$: 263.1283; found: 263.2364.

Supplementary data: ^{1}H NMR spectrum of *P. nigra* shoot resin, chromatograms of the RP-SPE fractions, results of the bioactivity assays and a compound overview with high resolution mass data can be found in supplementary data.

Acknowledgments - We wish to thank Dr Tamara Krügel and the greenhouse team of the Max Planck Institute for Chemical Ecology for growing *Populus nigra* plants.

References

[1] Escalante-Pérez M, Jaborsky M, Lautner S, Fromm J, Müller T, Dittrich M, Kunert M, Boland W, Hedrich R, Ache P. (**2012**) Poplar extrafloral nectaries: Two types, two strategies of indirect defenses against herbivores. *Plant Physiology*, ***159***, 1176-1191.

[2] Curtis JD, Lersten NR. (**1974**) Morphology, seasonal variation, and fluctuation of resin glands on buds and leaves of *Populus deltoides* (Salicaceae). *American Journal of Botany*, ***61***, 835-845.

[3] Escalante-Pérez M, Jaborsky M, Reinders J, Kurzai O, Hedrich R, Ache P. (**2012**) Poplar extrafloral nectar is protected against plant and human pathogenic fungus. *Molecular Plant*, ***5***, 1157-1159.

[4] Fathy HM, Aboushoer MI, Baraka A, Abdel-Kader MS, Omar AA. (**2009**) A new naphthoquinone with anti-inflammatory activity from an Egyptian collection of *Echiochilon fruticosum*. *Natural Product Sciences*, ***15***, 22-26.

[5] Salum ML, Robles CJ, Erra-Balsells R. (**2010**) Photoisomerization of ionic liquid ammonium cinnamates: one-pot synthesis-isolation of *Z*-cinnamic acids. *Organic Letters*, ***12***, 4808-4811.

[6] Delle Monache G, Botta B, Delle Monache F, Espinal R, De Bonnevaux SC, De Luca C, Botta M, Corelli F, Carmignani M. (**1993**) Novel hypotensive agents from *Verbesina caracasana*. 2. Synthesis and pharmacology of caracasanamide. *Journal of Medicinal Chemistry*, ***36***, 2956-2963.

[7] Li X, Wang Y, Wu J, Li Y, Wang Q, Xu W. (**2009**) Novel aminopeptidase N inhibitors derived from antineoplaston AS2-5 (Part II). *Bioorganic and Medicinal Chemistry*, ***17***, 3061-3071.

[8] Yavari I, Djahaniani H, Moradi L, Nasiri F. (**2005**) Triphenylphosphine-mediated serendipitous synthesis of alkyl cinnamates through the reaction of 3-hydroxy-4-methoxybenzaldehyde with dialkyl acetylenedicarboxylates. *Phosphorus, Sulfur, and Silicon and the Related Elements*, ***180***, 2149-2153.

[9] Sobolev VS, Horn BW, Potter TL, Deyrup ST, Gloer JB. (**2006**) Production of stilbenoids and phenolic acids by the peanut plant at early stages of growth. *Journal of Agricultural and Food Chemistry*, ***54***, 3505-3511.

[10] Hidalgo W, Schneider B. (**2015**) 4-Methoxycinnamic acid – an unusual phenylpropanoid involved in phenylphenalenone biosynthesis in *Anigozanthos preissii*. *Phytochemistry*, ***117***, 476 – 481.

[11] Merlani M, Barbakadze V, Amiranashvili L, Gogilashvili L, Yannakopoulou E, Papadopoulos K, Chankvetadze B. (**2010**) Enantioselective synthesis and antioxidant activity of 3-(3,4-dihydroxyphenyl)-glyceric acid- basic monomeric moiety of a biologically active polyether from *Symphytum asperum* and *S. caucasicu. Chirality*, ***22***, 717-725.

[12] Bankova V. (**1990**) Synthesis of natural esters of substituted cinnamic acids. *Journal of Natural Prod*ucts, ***53***, 821-824.

[13] Kartal M, Yildiz S, Kaya S, Kurucu S, Topcu G. (**2003**) Antimicrobial activity of propolis samples from two different regions of Anatolia. *Journal of Ethnopharmacology*, ***86***, 69-73.

[14] Uwai K, Osanai Y, Imaizumi T, Kanno S, Takeshita M, Ishikawa M. (**2008**) Inhibitory effect of the alkyl side chain of caffeic acid analogues on lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophages. *Bioorganic and Medicinal Chemistry*, ***16***, 7795-7803.

[15] Lee Y-T, Don M-J, Hung P-S, Shen Y-C, Lo Y-S, Chang K-W, Chen C-F, Ho L-K. (**2005**) Cytotoxicity of phenolic acid phenethyl esters on oral cancer cells. *Cancer Letters*, ***223***, 19-25.

[16] Wollenweber E, Asakawa Y, Schillo D, Lehmann U, Weigel, H. (**1987**) A novel caffeic acid derivative and other constituents of *Populus* bud excretion and propolis (bee-glue). *Zeitschrift für Naturforschung, C: Journal of Biosciences*, ***42***, 1030-1034.

[17] Greenaway W, Wollenweber E, Scaysbrook T, Whatley FR. (**1988**) Esters of caffeic acid with aliphatic alcohols in bud exudate of *Populus nigra*. *Zeitschrift für Naturforschung, C: Journal of Biosciences,* ***43***, 795-798.