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Antiproliferative activity of the Netherlands propolis and its active principles in cancer cell lines

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Abstract

The MeOH extract of the Netherlands propolis showed promising antiproliferative activity toward highly liver-metastatic murine colon 26-L5 carcinoma with an EC_{50} value of 3.5 μg/ml. Further, antiproliferative activity-guided purification of the MeOH extract led us to isolate four flavonoids (**1**–**4**), seven cinnamic acid derivatives (**5**–**11**) and two new glycerol derivatives (**12**, **13**), whose structures were elucidated on the basis of spectral analysis. The isolated compounds were tested for their antiproliferative activity against murine colon 26-L5, murine B16-BL6 melanoma, human HT-1080 fibrosarcoma and human lung A549 adenocarcinoma cell lines. The benzyl (**9**), phenethyl (**10**) and cinnamyl caffeates (**11**) possessed potent antiproliferative activities with EC_{50} values of 0.288, 1.76 and 0.114 μM, respectively, toward colon 26-L5 carcinoma. These caffeates were considered to be active constituents of the Netherlands propolis in their antiproliferative activity. The antioxidative activity of these caffeates may play an important role in their antiproliferative activities.

Keywords: Propolis; Antiproliferative activity; DPPH radical scavenging activity; Benzyl caffeate; Phenethyl caffeate; Cinnamyl caffeate

1. Introduction

Propolis is a resinous hive product collected by honeybees from various plant sources. It has a pleasant aromatic odor and yellow–green to dark brown color depending on its source and age (Ghisalberti, 1979). Propolis has a long history of being used in traditional medicine dating back at least to 300 BC (Ghisalberti, 1979) and has been reported to have a broad spectrum of biological activities, viz. anticancer, antioxidant, antiinflammatory, antibiotic, and antifungal activities (Banskota et al., 2001a; Burdock, 1998; Marcucci, 1995). It has recently gained popularity as a health drink and is used extensively in food and beverages in various parts of the world including Japan, the USA and Europe, where it is claimed to improve health and prevent diseases such as inflammation, heart disease, diabetes and even cancer. Due to these facts there is renewed interest in the composition and biological properties of propolis.

Propolis mainly contains sticky plant substances, collected by honeybees and mixed with bees wax and other bee secretions, thus, the composition of propolis other than wax absolutely depends on the vegetation of the area from where it was collected. Propolis from temperate zones contains predominantly phenolic compounds including flavonoids and cinnamic acid derivatives (Marcucci, 1995). Diterpenes and prenylated compounds, which are virtually absent in temperate propolis, on the other hand, were reported from the tropical propolis of the South-American continent together with lignans, flavonoids and other classes of compounds (Bankova et al., 2000). The difference in the composition of propolis from temperate and tropical zones is mainly due to their different vegetations. Even given this difference in their composition, propolis from both regions possessed similar biological properties (Burdock, 1998; Banskota et al., 2001a). In our previous work, we reported the isolation of 31 different consti-

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tuents from Brazilian propolis, of which three were new and 15 were isolated for the first time from propolis together with either their antiproliferative activity or hepatoprotective activity (Banskota et al., 2001b, 2000a, 1998; Basnet et al., 1996). Moreover, we also made a comparative study of propolis from different continents for their antiproliferative, antihepatotoxic and DPPH free radical scavenging activities (Banskota et al., 2000b). In the study, the MeOH extract of the Netherlands propolis showed promising antiproliferative activity. Thus, we conducted a chemical investigation of the MeOH extract of the Netherlands propolis. In this paper, we report the active principle of the Netherlands propolis together with its antiproliferative activities against different tumor cells.

2. Materials and methods

2.1. Chemicals

RPMI and Eagle's minimum essential medium (EMEM) were purchased from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan). 3-(4,5-Dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) was purchased from Sigma Chemicals (St. Louis, MO). Heat-inactivated fetal calf serum (FCS) was from Gibco BRL Products (Gaitherburg, MD). Coster polystyrene 96-well polystyrene plates (Corning Incorporated, Corning, NY) were used for the antiproliferative assay. Phenyl ethanol and cinnamyl alcohol were from Nacalai Tesque (Kyoto, Japan). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), sodium bicarbonate, glutamine, benzyl alcohol α-tocopherol, ascorbic acid and caffeic acid were from Wako Pure Chemical Industries (Osaka, Japan). 5-Fluorouracil was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan) and doxorubicin HCl was from Kyowa Hakko Co. Ltd. (Tokyo, Japan). Column chromatography was performed on silica gel 60 (Nacalai Tesque, Kyoto, Japan) and thin layer chromatography (TLC), both analytical and preparative, was carried out on percolated Merck Kieselgel 60 F_{254} and RP-18 F_{254} plates (0.25 or 0.50 mm thickness), respectively.

2.2. Instrumentation

UV absorptions were recorded on a Shimadzu UV-160A UV–visible spectrophotometer. Optical rotations were measured on a JASCO DIP-140 digital polarometer. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in $CHCl_3$ solution. FAB mass measurements were carried out on a JEOL JMS-700T spectrometer and glycerol was used as a matrix. ^{1}H-, ^{13}C- and 2D-NMR were recorded on a JEOL GX-400 spectrometer with tetramethylsilane (TMS) as an internal standard.

2.3. Propolis

The Netherlands propolis was collected in the northeast of the Netherlands in 1998, by scraping it off from the frames of beehives belonging to Honeybee Husbandry, Rutten, the Netherlands. The voucher specimen (No. TMPW 19920) is preserved in the Museum for Materia Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan, as a reference.

2.4. Extraction and Isolation

The powder of crude propolis (400 g) was extracted with MeOH (3L × 2) under reflux for 3 h to give a MeOH extract (210 g, 52.5%). The residue was further extracted with water (3L × 2) at 80 °C for 3 h. The filtrate was lyophilized to give a water extract (3.6 g, 0.9%) and the residue was discarded. The MeOH extract (150 g) was divided into ten fractions by silica gel column chromatography (50 × 7.5 cm) eluting with a $CHCl_3$/MeOH gradient system (fraction 1, 21.2 g; fraction 2, 2.9 g; fraction 3, 18.4 g; fraction 4, 6.6 g; fraction 5, 3.1 g; fraction 6, 5.0 g; fraction 7, 17.8 g; fraction 8, 4.8 g; fraction 9, 23.1 g and fraction 10, 42.0 g). Chrysin (**1**, 10.0 mg) and galangin 7-methyl ether (**2**, 12.5 mg) were obtained as precipitates from fractions 2 and 3, respectively. Further column chromatography followed by preparative TLC of fraction 6 gave **2** (659 mg), **5** (1.7 mg), **9** (11.0 mg) and **11** (86.3 mg), while fraction 7 gave **3** (7.7 mg), **4** (6.5 mg), **6** (6.9 mg), **8** (2.9 mg) and **9** (3.79 g). Similarly, fraction 8 gave **2** (14.2 mg), **4** (7.5 mg), **7** (46.8 mg), **12** (47.6 mg), **13** (10.3 mg) and a mixture of **9**–**11** (770 mg) in a ratio of 3.5:2:1, which were separated by reversed-phase preparative TLC (H_2O/MeOH/CH_3CN, 1:1:1).

2.4.1. 2-Acetyl-1,3-dicoumaroylglycerol (*12*)

A colorless amorphous solid; IR ($CHCl_3$) ν_{max} 3400, 1700, 1600, 1510, 1110 cm^{-1}; HRFABMS *m/z* 427.1355 [Calc. for $C_{23}H_{23}O_8$ $(M+H)^+$, 427.1393]: ^{1}H NMR (CD_3OD) δ 7.61 (2H, d, $J = 15.9$ Hz, H-3′, 3″), 7.47 (4H, d, $J = 8.6$ Hz, H-5′, 8′, 5″, 8″), 6.80 (4H, d, $J = 8.6$ Hz, H-6′, 9′, 6″, 9″), 6.33 (2H, d, $J = 15.9$ Hz, H-2′, 2″), 5.36 (1H, tt, $J = 6.0$, 4.4 Hz, H-2), 4.47 (2H, dd, $J = 12.0$, 4.1 Hz, H_2-1, 3), 4.35 (2H, dd, $J = 12.0$, 5.8 Hz, H_2-1, 3), 2.08 (3H, s, $COCH_3$); ^{13}C NMR (CD_3OD) δ 171.9 ($COCH_3$), 168.6 (C-1′, 1″), 161.3 (C-7′, 7″), 147 (C-3′, 3″), 131.3 (C-5′, 8′, 5″, 8″), 127.0 (C-4′, 4″), 116.8 (C-6′, 9′, 6″, 9″), 114.4 (C-2′, 2″), 71.0 (C-2), 63.3 (C-1, 3), 20.9 ($COCH_3$).

2.4.2. 2-Acetyl-1-coumaroyl-3-feruloylglycerol (*13*)

A colorless amorphous solid; $[\alpha]_D^{25}$ −6.5° ($c = 0.03$, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3400, 1700, 1670, 1600, 1510, 1430, 1370, 1260, 1160 cm^{-1}; HRFABMS *m/z* 457.1467

[Calc. for $C_{24}H_{25}O_9$ $(M+H)^+$, 457.1499]; ^{1}H NMR ($CDCl_3$) δ 7.64 (2H, d, $J=15.9$ Hz, H-3′, 3″), 7.40 (2H, d, $J=8.5$ Hz, H-5′, 8′), 7.07 (1H, dd, $J=8.3$, 1.7 Hz, H-9″), 7.03 (1H, d, $J=1.7$ Hz, H-5″), 6.91 (1H, d, J = 8.3 Hz, H-8″), 6.83 (2H, d, $J=8.5$ Hz, H-6′, 9′), 6.31 (1H, d, $J=15.9$ Hz, H-3″), 6.28 (1H, d, $J=15.9$ Hz, H-3′), 5.42 (1H, tt, $J=5.8$, 4.4 Hz, H-2), 4.47 (2H, $J=12.2$, 4.4 Hz, H_2-1, 3), 4.40 (2H, dd, $J=12.2$, 5.8 Hz, H_2-1, 3), 3.92 (3H, s, OCH_3), 2.09 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 171.9 ($COCH_3$), 166.9 (C-1′, 1″), 158.2 (C-7′), 148.2 (C-7″), 146.8 (C-6″), 146.0 (C-3″), 145.3 (C-3′), 130.4 (C-5′, 8′), 126.8 (C-4″), 126.7 (C-4′), 123.4 (C-8″), 115.9 (C-6′, 9′), 114.8 (C-5″), 114.4 (C-2″), 114.3 (C-2′), 109.4 (C-9″), 69.4 (C-2), 62.3 (C-1, 3), 56.0 (OCH_3), 20.9 ($COCH_3$).

2.5. Antiproliferative assay

Human HT-1080 fibrosarcoma (Rasheed et al., 1974), human lung A549 adenocarcinoma (Giard et al., 1973) and murine B16-BL6 melanoma (Hart, 1979) cell lines were maintained in EMEM medium supplemented with 10% FCS, 0.1% sodium bicarbonate and 2 mM glutamine. Murine colon 26-L5 carcinoma cell line (Ohnishi et al., 1997), on the other hand, was maintained in RPMI medium containing the same supplements as in EMEM. These are all highly metastatic cell lines except for A-549 carcinoma.

Cellular viability was determined using the standard MTT assay as reported previously (Banskota et al., 2000b; Rubinstein et al., 1990). In brief, exponentially growing cells were harvested and 100 μl of cell suspension containing 2000 cells was plated in 96-well microtiter plates. After 24 h of incubation to allow for cell attachment, the cells were treated with varying concentrations of test samples in medium (100 μl) and incubated for 72 h at 37 °C under 5% CO_2. Three hours after the addition of MTT, the amount of formazan formed was measured spectrophotometrically at 550 nm with a Perkin Elmer HTS-7000 plate reader. The test samples were first dissolved in DMSO and then diluted with medium; the final concentration of DMSO was less than 0.25%. Normal also had the same extent of DMSO. 5-Fluorouracil (5-FU) and doxorubicin HCl were used as positive controls, and EC_{50} values were calculated from the mean values of data from 4 wells.

2.6. DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the procedure described by Hatano et al. (1989). In brief, each different extract dissolved in EtOH or in water (500 μl) was mixed with an equal volume of DPPH solution (60 mM). The resulting solution was thoroughly mixed by vortex and the absorbance was measured at 520 nm after 30 min. The scavenging activity was determined by comparing the absorbance with that of the blank (100%) containing only DPPH and solvent.

3. Results

3.1. Extraction and isolation

The crude propolis was successively extracted with MeOH and water under reflux. The MeOH extract, having an interesting antiproliferative activity, was further fractionated into ten fractions by silica gel column chromatography. Chrysin (**1**, Wagner et al., 1976) and galangin 7-methyl ether (**2**) were obtained as precipitates from fractions 2 and 3, respectively. Fractions 6–8, having the strongest antiproliferative activity, were further subjected to chemical investigation. Three flavonoids, namely galangin 7-methyl ether (**2**), pinobanksin (**3**, Bohlmann et al., 1982) and pinobanksin 5-methyl ether (**4**, Bankova et al., 1983) were isolated from these fractions together with cinnamic acid (**5**), ferulic acid (**6**, Kelley et al., 1976), isoferulic acid (**7**, McCorkindale et al., 1969), 3,4-dimethoxycinnamic acid (**8**, de Silva et al., 1979), benzyl caffeate (**9**, Yamauchi et al., 1992), phenethyl caffeate (**10**, Grunberger et al., 1988) and cinnamyl caffeate (**11**) as shown in Fig. 1. The

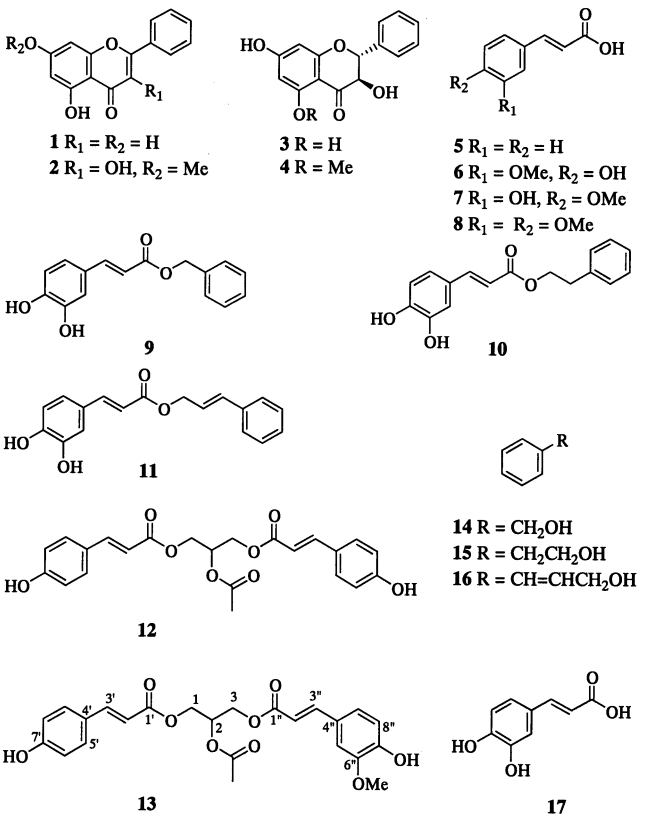

Fig. 1. Structure of the compounds tested for antiproliferative activity.

spectral data of these known compounds were identical to those in the literature or authentic samples. Moreover, two new glycerol derivatives, 2-acetyl-1,3-dicoumaroylglycerol (**12**) and 2-acetyl-1-coumaroyl-3-feruloylglycerol (**13**), were also isolated from fraction 8, whose structures were elucidated by spectral analysis including 2D NMR spectra.

3.2. Antiproliferative activity

The MeOH extract of the Netherlands propolis, having interesting antiproliferative activity (EC_{50}, 3.5 μg/ml), was divided into ten fractions by silica gel column chromatography and eluted with a $CHCl_3$/MeOH gradient system. The antiproliferative effects of these fractions were tested against murine colon 26-L5 carcinoma, and the EC_{50} values of fractions 1–10 were 20.7, 22.5, 37.5, 29.1, 7.1, 0.6, 0.8, 1.1, 6.6, 13.9 μg/ml, respectively. The active fractions, 6–8, gave 12 compounds (**2**–**13**), whose antiproliferative effects were tested against four different tumor cell lines. The EC_{50} values of all the isolated compounds together with benzyl alcohol (**14**), phenyl ethanol (**15**), cinnamyl alcohol (**16**) and caffeic acid (**17**) are summarized in Table 1. Benzyl (**9**), phenethyl (**10**) and cinnamyl (**11**) caffeates showed potent antiproliferative activity against HT-1080, colon 26-L5 and B16-BL6 cell lines with EC_{50} values of less than 14 μM, falling within the range of the potent cytotoxic agent ($EC_{50} < 4$ μg/ml) made by Geran et al. (1972). These caffeates also possessed the strongest antiproliferative effects toward A-549 among all the compounds. Flavanone derivatives **1** and **2** also possessed moderate antiproliferative activities toward HT-1080, colon 26-L5 and B16-BL6 melanoma cells among which the EC_{50} value of **1** toward colon 26-L5 carcinoma 13.4 μM (3.4 μg/ml) fell below the range of the potent cytotoxic agent made by Geran et al. (1972). Flavanols (**3**, **4**), on the other hand, showed only weak antiproliferative activities and other compounds [simple cinnamic acid derivatives **5**–**8**, caffeic acid (**17**) and alcohols **14**–**16**] possessed no antiproliferative activity, except for **16** (EC_{50} value, 47.8 μM against colon 26-L5; 44.0 μM against B16-BL6 melanoma). The water extract of the Netherlands propolis did not possess any antiproliferative effects toward colon 26-L5 carcinoma (Banskota et al., 2000b).

Table 1
Antiproliferative activity of the isolated compounds from the MeOH extract of the Netherlands propolis (EC_{50} values are in μM)

Compounds	Human		Murine	
	HT-1080	A-549	Colon 26-L5	B16-BL6
Chrysin (**1**)	17.3	> 200	13.4	20.5
Galangin 7-methyl ether (**2**)	26.3	> 200	30.3	20.8
Pinobanksin (**3**)	284	> 368	> 200	> 200
Pinobanksin 5-methylether (**4**)	> 200	> 200	> 200	187
Cinnamic acid (**5**)	> 200	> 200	> 676	> 200
Ferulic acid (**6**)	> 200	> 200	> 515	> 200
Isoferulic acid (**7**)	> 200	> 200	> 515	> 200
3,4-Dimethoxycinnamic acid (**8**)	> 200	> 200	> 200	> 200
Benzylcaffeate (**9**)	13.3	18.9	0.288	2.03
Phenethyl caffeate (**10**)	13.7	44.0	1.76	3.16
Cinnamyl caffeate (**11**)	9.45	18.9	0.114	1.92
Compound **12**	83.3	72.3	85.9	81.9
Compound **13**	80.5	> 200	75.5	66.0
Benzyl alcohol (**14**)	> 200	> 200	> 200	> 200
Phenyl ethanol (**15**)	> 200	> 200	> 200	> 200
Cinnamyl alcohol (**16**)	> 200	> 200	47.8	44.0
Caffeic acid (**17**)	> 200	> 200	167	> 200
5-Fluorouracil (5-FU)	3.92	5.76	0.538	4.69
Doxorubicin HCl (DOX)	0.086	0.189	0.017	0.012

3.3. DPPH radical scavenging activity

The DPPH radical scavenging activity of benzyl caffeate (**9**), phenethyl caffeate (**10**) and cinnamyl caffeate (**11**) was tested together with the well known antioxidants ascorbic acid and α-tocopherol. All these caffeates (**9**–**11**) possessed strong scavenging effects toward DPPH radical and their scavenging strengths were nearly equal to that of α-tocopherol and stronger than that of ascorbic acid (Fig. 2). Chrysin (**1**) and a new compound **12**, having mild antiproliferative effects, showed only weak DPPH radical scavenging activities (Fig. 2).

4. Discussion

Propolis, a complex mixture of plant metabolites, possesses a broad spectrum of biological activities including antibiotic, antioxidative, antiinflammatory and anticancer activities (Banskota et al., 2001a; Burdock, 1998; Marcucci, 1995). In our previous work, we found that the MeOH extract of the Netherlands propolis had interesting antiproliferative activity against highly metastatic liver murine colon 26-L5 carcinoma cells with an EC_{50} value of 3.5 μg/ml (Banskota et al., 2000b). Chemical examination of the MeOH extract was thus performed to identify the active components responsible for the antiproliferative activity of the Netherlands propolis. Eleven known compounds, either cinnamic acid derivatives or flavonoids (**1**–**11**) were isolated from the MeOH extract together with two new glycerol derivatives (**12**, **13**) through an antiproliferative activity-guided purification.

2-Acetyl-1,3-dicoumaroylglycerol (**12**) was isolated as a colorless amorphous solid with molecular formula $C_{23}H_{22}O_9$. The IR spectrum showed absorptions corresponding to hydroxyl (3400 cm^{-1}) and carbonyl groups

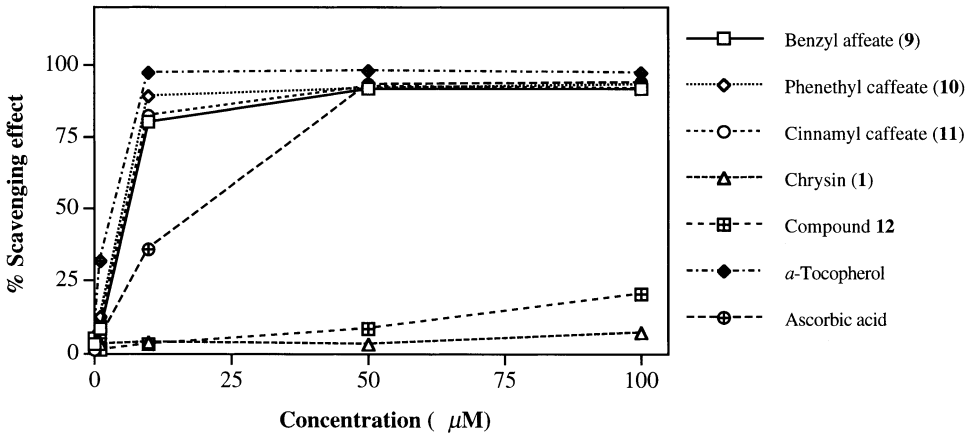

Fig. 2. The DPPH radical scavenging activity of the isolated compounds.

(1700, 1600 cm^{-1}). The ^1H and ^{13}C NMR spectra of **12** showed the signals of two equivalent coumaroyl groups together with two oxygenated methylenes, an oxygenated methine and an acetyl group. The ^1H–^1H COSY and HMQC correlations, together with the HMBC correlations depicted by arrows in Fig. 3, indicated that **12** is a symmetric glycerol ester having an acetyl group at C-2 and two *p*-coumaroyl group at C-1 and C-3. Thus, the structure of **12** was determined to be 2-acetyl-1,3-dicoumaroylglycerol.

2-Acetyl-1-coumaroyl-3-feruloylglycerol (**13**) was also isolated as a colorless amorphous solid with $[\alpha]_D^{25}$ -6.5° ($c = 0.03$, $CHCl_3$). The molecular formula of **13** was calculated as $C_{24}H_{24}O_9$ by FABMS, and its IR spectrum indicated the presence of hydroxyl as well as carbonyl groups. The ^1H and ^{13}C NMR spectra of **13** were found to be similar to those of **12**, except for a difference in one of the benzene rings. These spectral data indicated the presence of a feruloyl group at C-3 of **13** instead of two *p*-coumaroyl group as in **12**. This was confirmed by ^1H–^1H COSY, HMQC and HMBC correlations (Fig. 3). Thus, the structure of **13** was determined to be 2-acetyl-1-coumaroyl-3-feruloylglycerol.

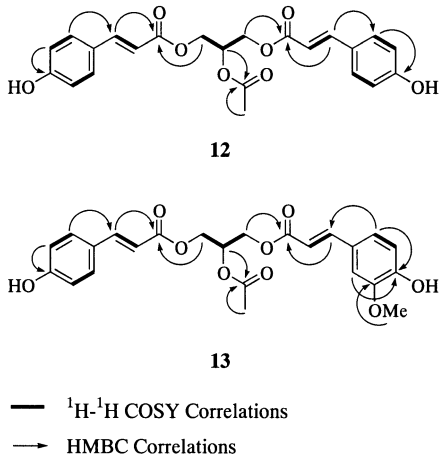

Fig. 3. The ^1H–^1H COSY and HMBC correlations of the new compounds.

Propolis from different continents was reported to have cytotoxic and antitumor effects (Burdock, 1998; Banskota et al., 2001a). The flavonoids, cinnamic acid derivatives including CAPE and artepillin C, and some diterpenoids were previously reported from propolis with interesting antitumor activity (Banskota et al., 2001a). In the present study, we further isolated cinnamic acid derivatives (**5**–**13**) and flavonoids (**1**–**4**) from the MeOH extract of the Netherlands propolis, which were previously reported from propolis of other regions (Marcucci, 1995). The benzyl (**9**), phenethyl (**10**) and cinnamyl caffeates (**11**) showed potent antiproliferative activities toward HT-1080 and B16-BL6 melanoma cell lines and most selectively toward murine colon colon 26-L5 carcinoma. Neither caffeic acid (**17**) nor its corresponding alcohols (**14**–**16**) showed any antiproliferative activity against the tested cell lines, except for **16** having moderate antiproliferative activity toward colon 26-L5 and B16-BL6 melanoma. These results indicate that the caffeates as a whole seem to be necessary for the antiproliferative effects. Moreover, compounds **9**–**11** were also found to be present in the highest content in the active fractions of the MeOH extract. Benzyl caffeate (**9**) was present at more than 3.7 g in fraction 7, while fraction 8 contained 770 mg of a mixture of **9**–**11** in the ratio of 3.5:1:2. From these facts, it was concluded that benzyl (**9**), phenethyl (**10**) and cinnamyl (**11**) caffeates are the active components responsible for the antiproliferative activity of the

Netherlands propolis. Of course, flavones **1** and **2** also possessed relatively stronger antiproliferative activities to all the cell lines other than A-549 cells, indicating that they might also partly contribute to the antiproliferative activity.

Phenethyl caffeate (**10**) is well know by the name caffeic acid phenethyl ester (CAPE) and was reported to have strong antitumor activity. In addition to in vitro cytotoxic activity (Grunberger et al., 1988; Lee et al., 2000), CAPE (**10**) has also been reported to decrease tumor formation in C57BL/6J-Min/+ mice bearing a germ line mutation in the Apc gene (Mahmoud et al., 2000). It has been reported to completely block the activation of NF-κB by tumor necrosis factor (Natarajan et al., 1996). In addition, CAPE (**10**) inhibited 5-lipoxygenase and soybean 15-lipoxygenase and completely blocked the production of ROS in human neutrophils and in the cell-free xanthine/XOD system (Mirzoeva et al., 1995). Thus, the antioxidative property of CAPE (**10**) seemed to play an important role in various biological systems. Benzyl caffeate (**9**) and cinnamyl caffeate (**11**) have similar chemical structures and possess stronger antiproliferative activities than CAPE (**10**) toward all the tested cell lines. Moreover, it is interesting to note here that both caffeates **9** and **11** showed stronger antiproliferative activities than 5-fluorouracil toward murine colon 26-L5 carcinoma and B16-BL6 melanoma and fell within the range of the potent cytotoxic agent ($EC_{50} < 4$ μg/ml) made by Geran et al. (1972). Benzyl caffeate (**9**) was previously isolated from Chinese propolis and has been reported to have strong antioxidative activity against autoxidation of methyl linoleate (Yamauchi et al., 1992). In the present study, we also observed an equal strength of scavenging activities of **9**–**11** toward DPPH radical to that of α-tocopherol, a well known antioxidant. Furthermore, these caffeates showed stronger DPPH radical scavenging activities than ascorbic acid used as positive control (Fig. 2). Thus, like CAPE (**10**), benzyl caffeate (**9**) as well as cinnamyl caffeate (**11**), appeared to be potent candidates for chemopreventive agents and their antioxidative activity may be associated with their antiproliferative properties. Further studies on these caffeates are in progress in our laboratory and will be reported elsewhere.

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