Indonesian Medicinal Plants. XXV.[1] Cancer Cell Invasion Inhibitory Effects of Chemical Constituents in the Parasitic Plant *Scurrula atropurpurea* (Loranthaceae)

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Six fatty acids (1—6), two xanthines (7, 8), two flavonol glycosides (9, 10), one monoterpene glucoside (11), one lignan glycoside (12), and four flavanes (13—16) were clarified by a bioassay-guided separation as chemical constituents of *Scurrula atropurpurea* (Loranthaceae), a parasitic plant of the tea plant *Thea sinensis* (Theaceae). Among these constituents, it was found that the alkynic fatty acid octadeca-8,10,12-triynoic acid (6) exhibits a more potent inhibitory effect on cancer cell invasion *in vitro* than flavanes [(+)-catechin (13), (−)-epicatechin (14), (−)-epicatechin-3-*O*-gallate (15) and (−)-epigallocatechin-3-*O*-gallate (16)].

Key words *Scurrula atropurpurea*; cancer cell invasion inhibition; octadeca-8,10,12-triynoic acid; catechin; Loranthaceae

Scurrula atropurpurea (BL.) DANS., a parasitic plant that attacks the tea plant *Thea sinensis* L., is called "benalu teh" in Indonesia and the whole plant (stems and leaves) has been traditionally used for the treatment of cancer in Java, Indonesia. So far, several research groups have carried out chemical and biological studies of Loranthaceous plants because several species in the family are well known as traditional medicines.[2] Herewith, we describe the bioassay-guided separation of chemical constituents of *Scurrula atropurpurea* and their *in vitro* inhibitory effects on cancer cell invasion.

The 70% aqueous acetone extract (9.5% yield from the dried plant) of the plant was partitioned into a mixture of ethyl acetate and water to give an ethyl acetate-soluble portion (2.1%) and a water-soluble portion (7.4%). The 70% aqueous acetone extract, ethyl acetate-soluble portion, and water-soluble portion showed cancer cell invasion inhibitory effects[3] of 32.4%, 67.3%, and 24.6%, respectively, at 10 μg/ml. The ethyl acetate-soluble portion was then separated by column chromatography to give four fractions (Fr-1, -2, -3, -4). Among the fractions, Fr-2 and Fr-3 exibited inhibitory effects, 82.4% and 53.2%, respectively, at 10 μg/ml, indicating that Fr-2 and Fr-3 contain the biologically active substances.

Fr-2 was purified by HPLC on normal adsorbent to afford six known fatty acids, namely (*Z*)-9-octadecenoic acid (**1**, 0.0171% yield from the plant),[4] (*Z*,*Z*)-octadeca-9,12-dienoic acid (**2**, 0.0041%),[4] (*Z*,*Z*,*Z*)-octadeca-9,12,15-trienoic acid (**3**, 0.0063%),[4] octadeca-8,10-diynoic acid (**4**, 0.0042%),[5,6] (*Z*)-octadec-12-ene-8,10-diynoic acid (**5**, 0.0082%),[7,8] and octadeca-8,10,12-triynoic acid (**6**, 0.017%).[7,8] From Fr-3, two known xanthines [theobromine (**7**, 0.0006%),[9,10] caffeine (**8**, 0.0103%)[10,11]], two known flavonol glycosides [quercitrin (**9**, 0.0202%),[12,13] rutin (**10**, 0.0051%)[12,14]], one known monoterpene glucoside [icariside B_2 (**11**), 0.0051%)[15]], one known lignan glycoside [aviculin (**12**, 0.0048%)[16]], and four known flavanes [(+)-catechin (**13**, 0.0106%),[14,17—20] (−)-epicatechin (**14**, 0.0001%),[12,14,18—20] (−)-epicatechin-3-*O*-gallate (**15**, 0.0006%),[18—20] and (−)-epigallocatechin-3-*O*-gallate (**16**, 0.0016%)[18—20]] were obtained by separation using an MCI GEL column and HPLC on reverse phase adsorbent. This is the first example of the isolation of alkynic fatty acids (**4**—**6**) from a Loranthaceous plant.

Next, the sixteen chemical constituents (**1**—**16**) were examined with respect to their effects on cancer cell invasion through a rat mesothelium monolayer using an MM1 cell line isolated from rat ascites hepatoma AH130 cells.[3]

The results of the assay revealed that except xanthines (**7**, **8**) and flavonol glycosides (**9**, **10**), all of the fatty acids (**1**—**6**), the monoterpene glycoside (**11**), the lignan glycoside (**12**), and the flavanes (**13**—**16**) exhibited inhibitory activity against cancer cell invasion. Octadeca-8,10,12-triynoic acid (**6**) in particular showed more potent inhibitory activity [10 μg/ml (37 μM); 99.4% inhibition, 5 μg/ml (18 μM); 94.9%] than the flavanes[21] [(+)-catechin (**13**), (−)-epicatechin (**14**), (−)-epicatechin-3-*O*-gallate (**15**), and (−)-epigallocatechin-3-*O*-gallate (**16**)] and other alkynic fatty acids (**4**, **5**). It should be noted that a rise in the number of unsaturation functions in the fatty acids seems to strengthen the inhibitory activity.

Meanwhile, flavanes are well known as the main con-

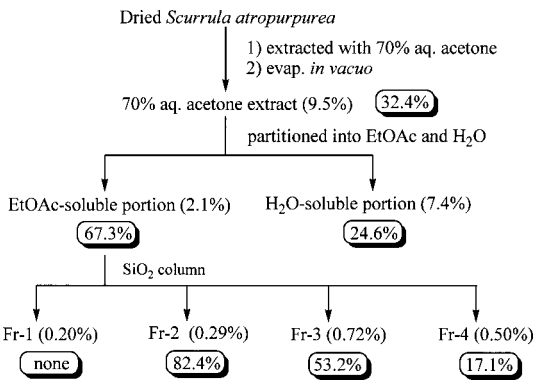

Fig. 1. Separation Procedure Guided by Inhibitory Effects on Cancer Cell Invasion

⬭ inhibitory rate at 10 μg/ml.

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stituents of the leaves of the tea plant and their anticancer activity,[22] including an inhibitory effect on cancer cell invasion,[21] has been noted. Accordingly, the stems and leaves of the host tree *Thea sinensis* were also extracted with 70% aqueous acetone and separated by the above-mentioned procedure for the parasitic plant, to yield four fatty acids (**1**, 0.0004%; **2**, 0.0011%; **3**, 0.0010%; **5**, 0.0030%), two xanthines (**7**, 0.0090%; **8**, 0.0180%), and six flavanes [(**13**, 0.0001%; **14**, 0.0200%; **15**, 0.161%; **16**, 0.638%), (+)-gallocatechin (**17**, 0.0004%)[18,20] and (−)-epigallocatechin (**18**, 0.0017%)[18—20]]. As expected,[21] (−)-epigallocatechin-3-*O*-gallate (**16**) is the major component and shows more potent activity [10 μg/ml (22 μM); 82.8%, 5 μg/ml (11 μM); 59.7%] than the other flavanes (**13**—**18**). However, we were unable to detect octadeca-8,10,12-triynoic acid (**6**) in the extract.

A summary of the results is as follows: 1) In the case of the parasitic plant *Scurrula atropurpurea*, the main biologically active substance for the treatment of cancer as a folk

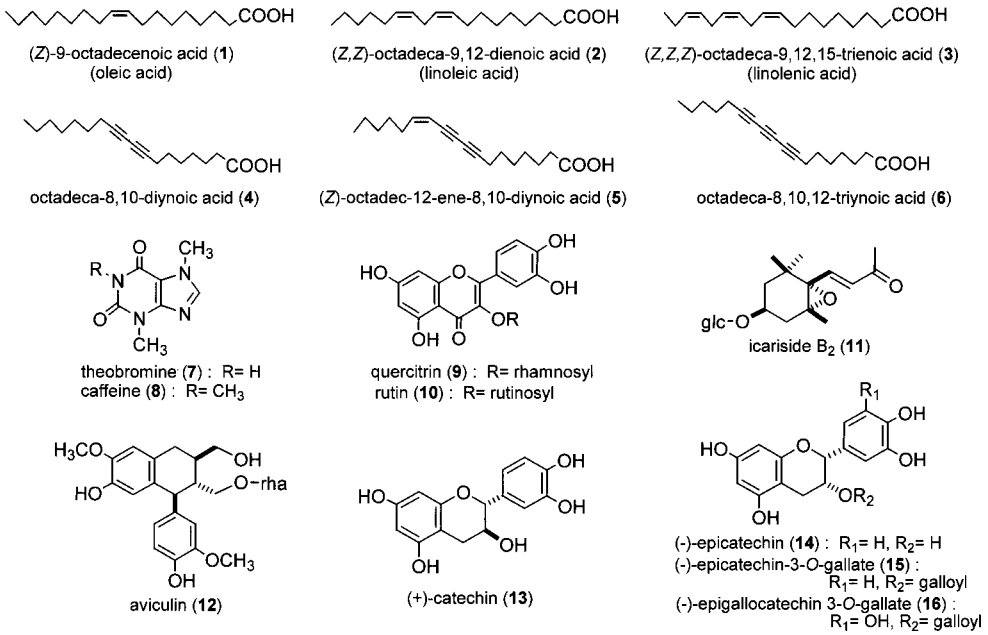

Fig. 2. Chemical Constituents of *Scurrula atropurpurea*

Table 1. Isolation Yields and Inhibitory Activities on Cancer Cell Invasion

Compound	Isolation yield (%)[a]		Inhibitory activity[b] (%)
	S. atropurpurea	*T. sinensis*	
Fatty acids			
(*Z*)-9-Octadecenoic acid (**1**)	0.0171	0.0004	13.0
(*Z,Z*)-Octadeca-9,12-dienoic acid (**2**)	0.0041	0.0011	15.7
(*Z,Z,Z*)-Octadeca-9,12,15-trienoic acid (**3**)	0.0063	0.0010	19.3
Octadeca-8,10-diynoic acid (**4**)	0.0042	—[d]	61.1
(*Z*)-Octadec-12-ene-8,10-diynoic acid (**5**)	0.0082	0.0030	89.8
Octadeca-8,10,12-triynoic acid (**6**)	0.0170	—[d]	99.4 (94.9)[c]
Xanthines			
Theobromine (**7**)	0.0006	0.0090	No activity
Caffeine (**8**)	0.0103	0.0180	No activity
Flavonol glycosides			
Quercitrin (**9**)	0.0202	—[d]	No activity
Rutin (**10**)	0.0051	—[d]	No activity
Monoterpene glucoside			
Icariside B (**11**)	0.0051	—[d]	19.4
Lignan glycoside			
Aviculin (**12**)	0.0048	—[d]	20.2
Flavanes			
(+)-Catechin (**13**)	0.0106	0.0001	34.0
(−)-Epicatechin (**14**)	0.0001	0.0200	20.3
(−)-Epicatechin-3-*O*-gallate (**15**)	0.0006	0.1606	59.9
(−)-Epigallocatechin-3-*O*-gallate (**16**)	0.0016	0.6380	72.8 (59.7)[c]
(+)-Gallocatechin (**17**)	—[d]	0.0004	24.2
(−)-Epigallocatechin (**18**)	—[d]	0.0017	27.8

a) Yield from the dried plant. *b*) Inhibitory rate at 10 μg/ml. *c*) Inhibitory rate at 5 μg/ml. *d*) Not determined.

medicine in Indonesia might be octadeca-8,10,12-triynoic acid (**6**). The flavanes (**13**—**16**) and alkynic fatty acids (**4**, **5**) may also be active substances used locally in Indonesia. 2) In the case of the host plant *Thea sinensis*, the flavanes (**13**—**18**) might be the main active principle group for the cancer cell invasion inhibitory effect, as is currently thought to be the case.[21)]

So far, ginsenoside Rg_3 which was isolated from red ginseng[23)] is the first naturally occurring material that exhibits a potent inhibitory effect on cancer cell invasion [25 μg/ml (32 μM); 98.8% inhibition]. Octadeca-8,10,12-triynoic acid (**6**), isolated here from *Scurrula atropurpurea*, seems to be the second example exhibiting potent activity.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-360 digital polarimeter and a cell length of 50 mm. FAB-MS and high resolution (HR)-FAB-MS were taken on a JMS 102 A mass spectrometer. IR spectra were run on a Shimadzu FT-IR 8500 spectrophotometer and UV spectra on a Hitachi U-3500 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-Lambda 500 spectrometer (500 MHz and 125.65 MHz, respectively). Chemical shifts are given in δ scale (ppm) relative to tetramethylsilane (δ=0 ppm) as internal standard. HPLC was done on a Tosoh PD-8020 and a Shimadzu LC-6A. Column chromatography was carried out on Silica gel 60 (230—400 mesh, Merck) and MCI GEL CHP20P (37—75 μ, Mitsubishi Chem. Co.). Thin-layer chromatography on Silica gel 60F_{254} plate (Merck) was used for checking the purity of the compounds. The spots were visualized by spraying the plates with 1% $Ce(SO_4)_2$ in 10% aqueous sulfuric acid followed by heating, or spraying with 0.33 M $FeCl_3$ solution.

Plant Materials *Scurrula atropurpurea* (Bl.) Dans. and *Thea sinensis* L. were collected at Puncak Pass, West Java, Indonesia in August 2001, and their scientific names were determined at the Herbarium Bogoriense, Research Centre for Biology-LIPI, Bogor, Indonesia.

Isolation of Chemical Constituents of *Scurrula atropurpurea* The stems and leaves of *Scurrula atropurpurea* (500 g) were extracted four times with 70% aqueous acetone for 24 h at room temperature. The combined extract solution was evaporated under reduced pressure to give a 70% acetone extract (47.5 g, 9.5% from the plant). The 70% aqueous acetone extract was partitioned into ethyl acetate and water (1 : 1) to give an ethyl acetate-soluble portion (10.5 g, 2.1%) and a water-soluble portion (37.0 g, 7.4%). The ethyl acetate-soluble portion (5.0 g) was subjected to silica gel column chromatography (SiO_2 375 g, eluting with hexane–ethyl acetate, chloroform–methanol, chloroform–methanol–water, then methanol) to afford Fr-1 (475 mg, 0.20%), Fr-2 (690 mg, 0.29%), Fr-3 (1.72 g, 0.72%), and Fr-4 (1.19 g, 0.50%). Fr-2 was purified by HPLC (Wakosil 5SIL, hexane–ethyl acetate) to give (*Z*)-9-octadecenoic acid (**1**, 40.7 mg, 0.0171%, from the plant),[4)] (*Z*,*Z*)-octadeca-9,12-dienoic acid (**2**, 9.8 mg, 0.0041%),[4)] (*Z*,*Z*,*Z*)-octadeca-9,12,15-trienoic acid (**3**, 15.0 mg, 0.0063%),[4)] octadeca-8,10-diynoic acid (**4**, 10.0 mg, 0.0042%),[5,6)] (*Z*)-octadec-12-ene-8,10-diynoic acid (**5**, 19.5 mg, 0.0082%),[7,8)] and octadeca-8,10,12-triynoic acid (**6**, 40.5 mg, 0.017%).[7,8)] Furthermore, Fr-3 was separated by MCI GEL CHP20P (400 g) column chromatography (eluting with methanol–water) followed by HPLC (CAPCELL PAK C18 UG120, acetonitrile–water) to obtain theobromine (**7**, 1.5 mg, 0.0006%),[9,10)] caffeine (**8**, 24.5 mg, 0.0103%),[10,11)] quercitrin (**9**, 48.0 mg, 0.0202%),[12,13)] rutin (**10**, 12.2 mg, 0.0051%),[12,14)] icariside B_2 (**11**, 12.1 mg, 0.0051%),[15)] aviculin (**12**, 11.5 mg, 0.0048%),[16)] (+)-catechin (**13**, 25.3 mg, 0.0106%),[14,17—20)] (−)-epicatechin (**14**, 0.2 mg, 0.0001%),[12,14,18—20)] (−)-epicatechin-3-*O*-gallate (**15**, 1.5 mg, 0.0006%),[18—20)] and (−)-epigallocatechin-3-*O*-gallate (**16**, 3.8 mg, 0.0016%).[18—20)] The chemical structures of the isolated compounds were confirmed by comparing the physicochemical properties with those in the literature and authentic samples.

Isolation of Chemical Constituents of *Thea sinensis* The stems and leaves of *Thea sinensis* (500 g) were extracted four times with 70% aqueous acetone for 24 h at room temperature. The combined solution was evaporated under reduced pressure to give the 70% aqueous acetone extract (50.1 g, 10.0% yield from the plant), which was partitioned into a mixture of ethyl acetate and water (1 : 1). Each portion was concentrated to give an ethyl acetate-soluble portion (11.0 g, 2.2%) and a water-soluble portion (38.5 g, 7.7%). The ethyl acetate-soluble portion (5.0 g) was separated by silica gel column chromatography (375 g), in the same manner as for *Scurrula atropurpurea*, to give Fr-I (410 mg), Fr-II (630 mg), Fr-III (2.33 g), and Fr-IV (1.49 g). Further separation of Fr-II by normal phased HPLC gave (*Z*)-9-octadecenoic acid (**1**, 0.9 mg, 0.0004%), (*Z*,*Z*)-octadeca-9,12-dienoic acid (**2**, 2.5 mg, 0.0011%), (*Z*,*Z*,*Z*)-octadeca-9,12,15-trienoic acid (**3**, 2.3 mg, 0.0010%), and (*Z*)-octadec-12-ene-8,10-diynoic acid (**5**, 6.8 mg, 0.0030%), while separation of Fr-III by MCI GEL CHP20P column chromatography followed by reverse phase HPLC afforded theobromine (**7**, 20.5 mg, 0.0090%), caffeine (**8**, 40.9 mg, 0.0180%), (+)-catechin (**13**, 0.2 mg, 0.0001%), (−)-epicatechin (**14**, 45.4 mg, 0.0200%), (−)-epicatechin-3-*O*-gallate (**15**, 365 mg, 0.161%), (−)-epigallocatechin-3-*O*-gallate (**16**, 1.45 g, 0.638%), (+)-gallocatechin (**17**, 0.9 mg, 0.0004%),[18,20)] and (−)-epigallocatechin (**18**, 3.9 mg, 0.0017%).[18—20)]

Cell Lines and Culture Rat mesothelial cells were isolated from Donryu rat (Japan SLC, Inc., Hamamatsu, Japan) mesentery and cultured in minimum essential medium (MEM) containing 2-fold amino acids and vitamins supplemented with 10% FCS as reported previously.[3)] The MM1 cell line, which is a highly invasive clone isolated from parental rat ascites hepatoma AH130 cells, was cultured in suspension in MEM containing 2-fold concentrated amino acids and vitamins supplemented with 10% FCS. Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Invasion Assay MM1 cells (2×10^5 cells) were seeded over a rat mesothelial cell monolayer (MCL) and cultured in medium containing the materials to be tested. Invasion experiments were started by the addition of 25 μM 1-oleoyl-lysophosphatidic acid (LPA). After five hours, the supernatant was removed and the resultant monolayer was fixed *in situ* with 10% formalin. The number of penetrated single tumor cells and tumor cell colonies (collectively called invasion foci) was counted under a phase-contrast microscope. The activity was expressed as an inhibitory rate (%) calculated from the number of invasion foci.

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