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The Chemical Constituents of *Ficus benjamina* Linn. and Their Biological Activities

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ABSTRAK

Daun-daun, kulit kayu dan buah-buah *Ficus benjamina* Linn. didedahkan kepada pengekstrakan dan pengasingan menggunakan teknik-teknik kromatografik untuk menghasilkan enam sebatian (asid sinamik, laktosa, "naringenin", "quercetin", asid kafeik dan "stigmasterol". Struktur-struktur sebatian tersebut ditentukan oleh teknik-teknik spektroskopik dan perbandingan dengan data yang diterbitkan. Sebatian-sebatian tersebut ditutupi untuk aktiviti antimikrobia ke atas dua spesies bakteria (*Bacillus cereus* dan *Pseudomonas aeruginosa*) dan aktiviti sitotoksik terhadap garis sel "T-lymphoblastic leukemic (CEM-SS). Asid kafeik menunjukkan aktiviti sitotoksik yang kuat dengan nilai IC sebanyak 25 mg/mL.

ABSTRACT

The leaves, bark and fruits of *Ficus benjamina* Linn. were subjected to extraction and isolation using chromatographic techniques to yield six compounds (cinnamic acid, lactose, naringenin, quercetin, caffeic acid and stigmasterol). The structures of the compounds were determined by spectroscopic techniques and by comparison with published data. The compounds were screened for antimicrobial activity against two species of bacteria (*Bacillus cereus* and *Pseudomonas aeruginosa*) and cytotoxic activity against T-lymphoblastic leukemic (CEM-SS) cell line. Caffeic acid exhibited strong cytotoxic activity with IC_{50} value of 25 mg/mL.

Keywords: *Ficus benjamina*, naringenin, quercetin, antimicrobial, cytotoxicity

INTRODUCTION

Ficus benjamina Linn. belonged to the family Moraceae (Burkill 1966). It is a medium-sized tree with several spreading branches from the base. It is widely distributed in the tropics (Baily 1963). The leaves are 2-5 cm wide and the bark is pale brown or greyish brown. The plant is known locally as "beringin, waringin and jejawi" (Holttum 1969). It is traditionally used as a stomachic, hypotensive and anti-dysentery agent (Trivedi *et al.* 1969). Previous studies on *Ficus* species revealed the presence of several compounds such as alkaloids (Beat *et al* 1990), triterpenes (Mohammad *et al.* 1991), ascorbic acid. (Ikhlas *et*

al. 1993) and flavonoids (Ilyas and Ilyas 1990). This paper reports on the isolation and identification of six compounds from the plant and their antimicrobial and cytotoxic activities. There has been no previous report on the chemical constituents and biological activity of *Ficus benjamina.*

GENERAL EXPERIMENTAL

Melting points were determined on a Kofler hot-stage and were uncorrected. IR spectra were recorded on a Perkin-Elmer FTIR 1725X. UV spectra were recorded on a Shimadzu UV-2100 spectrophotometer ^{1}H and ^{13}C NMR spectra were recorded on JOEL 400 MH_z spectrometer using TMS as internal standard in CD_3COCD_3 and DMSO. Mass spectra were obtained using a Finnigan Mat Model SSQ 710 spectrometer.

Plant Material

The leaves, bark and fruits of *Ficus benjamina* were collected from Universiti Putra Malaysia campus in February 1999 and a voucher specimen (10448) was deposited at the Herbarium of the Department of Biology, UPM.

Extraction and Isolation of Compounds

Air dried leaves, bark and fruits of *Ficus benjamina* were extracted successively with petroleum ether, chloroform and methanol. The extracts were concentrated under vacuum. The chloroform extract of the leaves (48.0 grams) was subjected to column chromatography using gradient solvent mixtures; 100% petroleum ether, petroleum ether/chloroform (2:5), chloroform/methanol (8:3) and 100% methanol to yield 15 fractions. Fractions 8 and 9 were further rechromatographed with the chloroform/methanol (8:3) to yield compound (I) as a white powder. Rechromatographing of fractions 10-13 yielded compound (II) as a white powder.

The methanol extract (164.3 grams) was redissolved in 95% aqueous methanol (500 mL) and then reextracted with n-hexane (500 mL). The methanol layer was concentrated, suspended in water (1 L) and then reextracted twice with 500 mL of chloroform, ethyl acetate and n-butanol successively. The n-butanol extract was subjected to column chromatography eluted with 100% chloroform with an increasing amount of methanol to give 18 fractions. Fractions 3-9 were recombined and chromatographed using chloroform/methanol (1:1) gradient of increasing polarity followed by recrystallisation from methanol to yield compound (III). Fractions 10-18 were also recombined and eluted with chloroform/methanol (5:2) to yield a yellow powder which was recrystallised from methanol to afford compound (IV).

A portion of the n-butanol fraction of the methanol extracts (8.0 g) of the fruits of *Ficus benjamina* was eluted with 100% chloroform, chloroform/methanol (7:3) and 100% methanol to give 16 fractions. Fractions 3-10 were concentrated and analysed by TLC to give white solid after washing with ethanol repeatedly

and recrystallised from ethanol to give compound (V). However, the chloroform extract of the bark (10.0 g) yielded only one compound (VI).

Antimicrobial Assay

The microorganisms were obtained from the culture collection of the Department of Biotechnology, UPM. The stock cultures were grown on potato dextrose agar (PDA) for 24 h at 28°C at which time the cells were harvested by centrifugation (4°C, 2000 rpm, 3 min.). The cells were washed and suspended in sterile 0.9% saline to give a final concentration of 10^5-10^6 CFU/mL using a haemocytometer (Berdy 1985; Bergeys 1957). The microbial strains used were: *Bacillus cereus* NRRLUI-1447, *Pseudomonas aeruginosa* UI-60690; *Aspergillus ochraceus* NRRL 398, *Candida lipolytica* ATCC 2075, *Sacchromyces cereviseae* NRRL 2034 and *Sacchromyces alipolytica.*

Antimicrobial activity of the isolated compounds were tested using disc diffusion method according to Bauer *et al.* (1966). The discs were prepared by impregnating them in ethanolic solution of each sample (10 mg/mL). They were then evenly spaced out on the agar surface previously inoculated with the suspension of each microorganism to be tested. Standard discs of nystatin (50 g/discs) and streptomycin sulphate (25 g/discs) were used as positive controls. The plates were incubated at 37°C for 24 h and the antimicrobial activity was recorded by measuring the diameter of the clear inhibition zones around each disc.

Cytotoxity Test

The cytotoxic effect of the compounds was evaluated on (CEM-SS) T-lymphoblastic leukemic cell line which was obtained from the National Cancer Institute, Maryland, USA and maintained as previously described (Weilow *et al.* 1989). Cell viability was determined by using the microtitration assay (Ali *et al.* 1998). Cytotoxicity was recorded as the 50% inhibition concentration (IC_{50}) with reference to the untreated positive control cells (Ali *et al.* 1997).

RESULTS AND DISCUSSION

Extraction of the leaves, bark and fruits of *Ficus benjamina* followed by extensive column chromatography resulted in the isolation of cinnamic acid, lactose, naringenin, quercetin, caffeic acid and stigmasterol.

Compound (I) was obtained as white powder (24.9 mg) from chloroform extract of the leaves of *Ficus benjamina* with melting point 128-130°C (Buckingham 1994, 130-131°C). The aromatic regions in ^{1}H-NMR spectrum indicated the presence of five protons at d 7.83, 7.56, 7.42, 7.36, 7.24 each was assigned to proton in positions-3, 4, 5, 6 and 2 respectively. The other two signals which appeared at d 6.49 and 6.43 indicated the presence of two protons at C-7 and C-8. The presence of seven signals in the ^{13}C-NMR spectrum indicated the presence of nine carbons in the molecule which include seven methine carbons at 117.8, 147.5, 128.8 (×2), 134.5 and 129.4(×2) ppm assigned to C-2, C-3,

C-5 and C-6 (overlapped), C-4, C-7 and C-8 (overlapped) respectively. The two quartenary carbons at 131.2 and 173.2 ppm were assigned to C-1 and C-9. The mass spectrum indicated the presence of a molecular ion peak at m/z 148 which corresponded to the molecular formula $C_9H_8O_2$ with a base peak at m/z 66. Loss of one molecule of water was indicated by the fragment at m/z 130. Comparison of these spectral data with those reported previously (Byung *et al.* 1996) supported that Compound (1) is cinnamic acid.

Lactose (II) was isolated as white powder with melting point 229-230°C (Tadasu *et al.* 1985, 230-232°C). The chemical test and the spectral data obtained for these compounds were in agreement with published data (William 1987).

Naringenin (III) was obtained from the n-butanol fraction of the methanol extract of the leaves of *Ficus benjamina* as white powder with melting point 249-252°C (Buckingham 1994, 250-251°C). The ^{1}H-NMR spectrum indicated the presence of two hydroxyl groups which appeared as broad singlets at δ 9.73 and δ 8.63 assigned to 4′-OH and 7-OH respectively. The other hydroxyl group appeared as a sharp singlet at δ 12.18 indicative of a chelated hydroxyl proton at C-5. A doublet of a doublet at d 7.41 (J=1.9 H_z) was assigned to the two equivalent protons at H-3′ and H-5′ respectively. The other two equivalent protons which also appeared as a doublet of a doublet at δ 6.92 (J=1.9 H_z) was assigned to protons at H-2′ and H-6′. Another two protons which appeared as a pair of doublets at δ 5.96 (J=1.9 H_z) were assigned to the two protons at C-6 and C-8. A doublet at δ 5.42 (J=2.9 H_z) was assigned to proton at C-2 which is coupled to the methylene protons at H-3. The signal for H-3a appeared at d 2.74 as a doublet of a doublet with coupling constant values of 3.2 H_z ($J\,H_{3a\text{-}2}$) and 3.0 H_z ($J\,H_{3a\text{-}3b}$). The H-3b proton gave a signal at δ 3.27 with coupling constant values of 4.2 H_z ($J\,H_{3b\text{-}2}$) and 2.9 H_z ($J\,H_{3b\text{-}3a}$). The ^{13}C-NMR spectrum assignments were made by DEPT experiment and also by comparison with literature values (Eberhaed and Wolfgang 1987; Agrawal 1989). The spectrum showed the presence of fifteen carbons consisting of seven quartenary carbons at 197.2, 167.3, 165.5, 164.6, 158.7, 130.8 and 103.2 ppm which were assigned to C-4, C-7, C-5, C-9, C-4′, C-1′ and C-10 respectively. The seven methine carbons were at 129.0 (×2), 116.2 (×2), 95.8, 96.8 and 79.9 ppm assigned to C-3′ & C-5′, C-2′ & C-6′, C-6, C-8 and C-2 respectively. The methylene carbon for C-3 appeared at 43.5. Mass spectrum gave a molecular ion at 272. This is consistent with the presence of three hydroxyl groups and a molecular formula of $C_{15}H_{12}O_5$. Based on the spectral data obtained and compared with published data (Barakat *et al.* 1999), Compound III concluded to be naringenin.

Quercetin (3,5,7,3′,4′-pentahydroxyflavone) Compound (IV) was isolated from the n-butanol fraction of the methanol extract of the leaves of *Ficus benjamina* as yellow needles with melting point 296-298°C (Uphof 1968, 298-300°C). The ^{1}H-NMR spectrum of compound (IV) showed peaks at δ 9.36. These are characteristics of a 3′ and 4′-disubstituted B-ring of flavonol (Mabry *et al.* 1970; Banerji *et al.* 1969). The two broad singlets at δ 10.86 and 9.66 may be attributed to the two hydroxyl groups at C-7 and C-3 respectively, whereas

the hydroxyl group at position–5 appeared at very low field as a sharp singlet at δ 12.52 due to chelation to the carbonyl group. The aromatic region in the ^{1}H-NMR spectrum showed the presence of five protons each. A pair of doublets at δ 6.48 and 6.22 each with coupling constant 2.2 H_z due to *meta* coupling were assigned to protons H-8 and H-6, respectively. Another doublet was observed at δ 7.71 with coupling constant 2.2 H_z assigned to H-2′. Proton at H-6′ occurred as doublet of a doublet at δ 7.57 with coupling constant 2.2 H_z. The doublet at d 6.95 with coupling constant 8.5 H_z was assigned to proton at H-5′. The ^{13}C-NMR spectrum indicated the presence of fifteen carbon atoms including ten quartenary carbons at 146.9, 135.8, 175.9, 156.2, 163.9, 160.8, 103.1, 122.1, 145.1 and 147.8 ppm assigned to C-2, C-3, C-4, C-5, C-7, C-9, C-10, C-1′, C-3′ and C-4′ respectively. The five methine carbons occurred at 98.3, 93.4, 115.1, 115.7 and 120.1 ppm assigned to C-6, C-8, C-2′, C-5′ and C-6′ respectively. This was supported by the results obtained from DEPT experiment. The mass spectrum gave a molecular ion peak at m/z 302 which is consistent with the molecular formula of $C_{15}H_{10}O_7$. The structural assignment of this compound was supported by m.p., IR, UV, ^{1}H, ^{13}C-NMR, DEPT and comparison with published data (Buckles 1962).

Caffeic acid (V) was isolated as light yellow powder (25.5 mg), with melting point 195-198°C (Kiritsakis 1998, 200°C). The ^{1}H-NMR spectrum revealed the presence of two doublets at δ 6.80 and 6.95 each with coupling constants of 8.1 H_z assigned to the two protons at C-5 and C-6 respectively. Another aromatic proton on positin H-2 occurred as sharp singlet at δ 7.05. The two methine protons at positions H-7 and H-8 appeared as two doublets at δ 7.57 (J=15.8 H_z) and 6.21 (J=15.8 H_z), whereas the broad singlet peaks at δ 4.95 indicated the presence of two hydroxyl groups in positions 3 and 4.

The ^{13}C-NMR spectrum showed the presence of nine carbons in the spectrum in which five were methine carbons (three aromatic carbons and two aliphatic carbons) at 117.4, 116.0, 123.8, 148.0 and 116.4 ppm assigned to C-2, C-5, C-6, C-7 and C-8 respectively. The four quartenary carbons at 128.7, 147.6, 150.3 and 172.0 ppm were assigned to carbon signals at positions C-1, C-3, C-4 and C-9 respectively. The mass spectrum indicated the presence of a molecular ion as the base peak at m/z 180 which corresponds to the molecular formula $C_9H_8O_4$. Loss of one hydroxyl group from the molecular ion gave a peak at m/z 163 and subsequent loss of another water molecule from this fragment ion gave a fragment at m/z 145. Thus the compound was identified as caffeic acid (3, 4-dihydroxy cinnamic acid).

Stigmasterol (VI) was obtained from chloroform extract of the bark of *Ficus benjamina* as white powder with melting point 166-167°C (Schwartz and Wall 1995, 166-168°C). Based on the spectral data and on comparison with literature values (Cechinel *et al.* 1998), the compound was identified as stigmasterol.

Antibacterial screening on the compounds showed that quercetin and naringenin exhibited the stronger antibacterial activity. Cinnamic acid and lactose showed strong activity against the Gram-negative and moderate activity against the Gram-positive bacteria. Caffeic acid and stigmasterol yield weak

activity against the two species of bacteria. However, all the compounds did not show inhibitory activity against the four species of fungi. They showed negative cytotoxicity effect, except for caffeic acid, which exhibited strong cytotoxic activity against T-lymphoblastic leukemic (CEM-SS) cell line with an IC_{50} value of 25 mg/mL (Table 1 and *Fig. 1*).

The cytotoxic effect of caffeic acid at the inhibition concentration (IC_{50}) value as compared with control is shown in *Fig. 2* in which the cells became granulated and fragmented.

TABLE 1
Antimicrobial activity of compounds isolated (concentration 100 µg/ml, methanol) from *Ficus benjamina*

compound	bacteria		fungi			
	B. cereus	*P. aeruginosa*	*A. ochraceous*	*C. lipolytica*	*S. cereviseae*	*S. lipolytica*
cinnamic acid	++	++	-	-	-	-
lactose	+	+	-	-	-	-
naringenin	+++	+++	-	-	-	-
quercetin	+++	+++	-	-	-	-
caffeic acid	+	+	-	-	-	-
stigmasterol	++	++	-	-	-	-

B. = *Bacillus* *P.* = *Pseudomonas* *A.* = *Aspergillus* *C.* = *Candida*
S. = *Sacchromyces* - no inhibition (0 mm) + weak inhibition (1-9 mm)
++ medium inhibition (10-14 mm) +++ strong inhibition (15-19 mm)

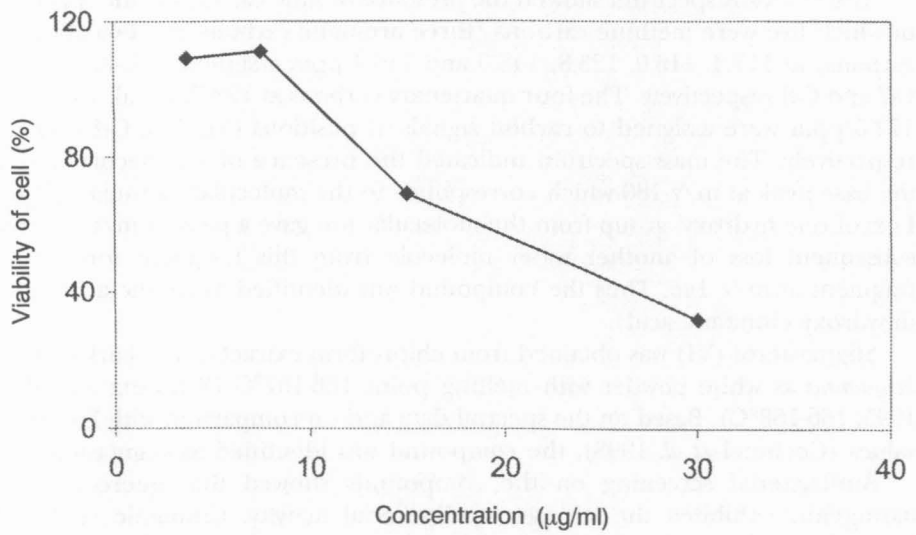
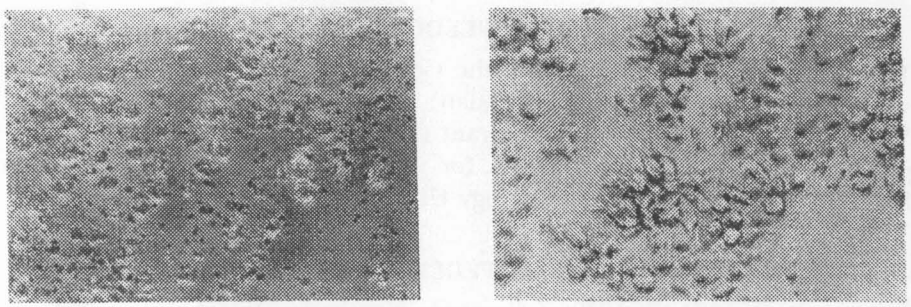
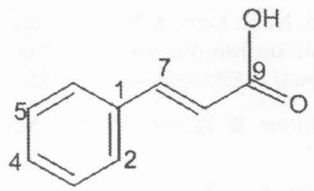


Fig. 1. Percentage viability of (CEM-SS) cells after being treated with different concentrations of caffeic acid

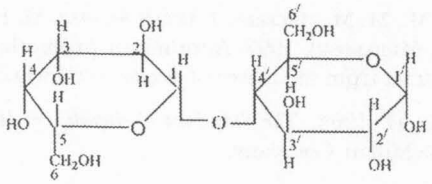


(a) (b)

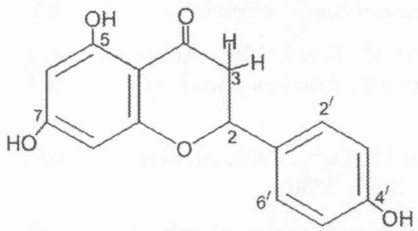
Fig. 2: Morphology of (CEM-SS) cells line treated for 72 hours
(a) 15 (g/ml of caffeic acid (b) control



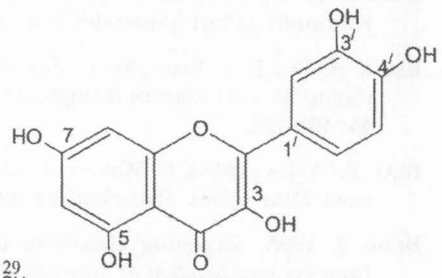
(I)



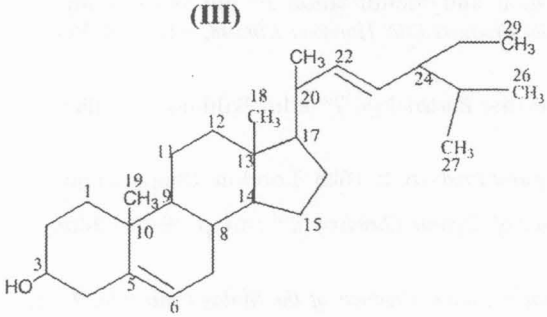
(II)



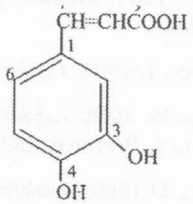
(III)



(IV)



(VI)



(V)

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