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Investigation on the Chemical Constituents of the Leaves of *Ficus elastica* Roxb. and Their Antimicrobial Activity

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ABSTRAK

Empat sebatian (emodin, sukrosa, morin dan rutin) diasingkan daripada daun-daun *Ficus elastica* Roxb. Struktur-struktur sebatian tersebut dibentuk menggunakan teknik-teknik spektroskopik dan perbandingan dengan data yang diterbitkan. Sebatian-sebatian tersebut ditutup untuk aktiviti antimikrobia ke atas dua spesies bakteria, *Bacillus cereus* (Gram-positif) dan *Pseudomonas aeruginosa* (Gram-negatif) dan empat spesies fungus dengan menggunakan kaedah resapan cakera. Sebatian-sebatian itu menunjukkan aktiviti antibakteria tetapi tiada aktiviti antifungus diperhatikan ke atas organisma-organisma yang diuji.

ABSTRACT

Four known compounds (emodin, sucrose, morin and rutin) were isolated from the leaves of *Ficus elastica* Roxb. The structures of the compounds were established by spectroscopic techniques and by comparison with published data. The compounds were screened for antimicrobial activity against two species of bacteria, *Bacillus cereus* (Gram-positve) and *Pseudomonas aeruginosa* (Gram-negative) and four species of fungi by using the disc diffusion method. The compounds showed antibacterial activity but no antifungal activity was observed against the tested organisms.

Keywords: *Ficus elastica*, emodin, morin, antimicrobial, cytotoxicity

INTRODUCTION

Many *Ficus* species are commonly used in traditional medicine to cure various diseases. They have long been used in folk medicine as astrigents carminatives, stomachics, vermonicides, hypotensives, antihelmintics and anti-dysentery drugs (Trivedi *et al.* 1969). Previous phytochemical studies on the genus showed the presence of flavonoids (Mohammad 1991), alkaloids (Ikhlas *et al.* 1993), organic acids (Ilyas 1990) and triterpenes (Beat 1990).

Ficus elastica (Moraceae) is a widely-spread evergreen tree up to 30 m tall. The leaves are 7-20 cm long, with smooth edges and blunt pointed tips. The leaves are about a foot long and are thick with deep green colour. The plant is known locally as "india-rubber tree" (Burkill 1966). No chemical and biological investigations have been reported on *Ficus elastica*. In this study we report the

chemical constituents and antimicrobial activity of the compounds isolated from the plant.

MATERIAL AND METHODS

Plant Material

The leaves of *Ficus elastica* were collected from the Universiti Putra Malaysia campus in April 2000 and identified by taxonomist Umi Kalsom Yusuf from UPM. A voucher specimen (16291) was deposited at the Herbarium of the Department of Biology, UPM.

General Material and Methods

Melting points were determined on a Kofler hot-stage microscope melting point apparatus and were uncorrected. IR and UV spectra were obtained on Perkin-Elmer lambda model 1330 and 20 spectrometers respectively. ^{1}H and ^{13}C-NMR were recorded on JEOL JNM-GSX 400 spectrometer. Mass spectra were obtained using GCMS-QP 5050 A Shimadzu mass spectrometer. Column chromatography and analytical TLC were carried out using silica gel, Merck 230 - 400 mesh and Merck 70-230 mesh.

Extraction and Isolation of Compounds

The leaves of *Ficus elastica* were air-dried, ground and subjected to extraction with petroleum ether, chloroform and methanol successively. The solvents were then removed under reduced pressure to give the following extracts as shown in Table 1.

TABLE 1
The extracts of the leaves of *Ficus elastica*

Material (g)	Solvent	Colour and nature of extracts	Yield of extracts (g)
Leaves (2500)	petroleum ether	yellowish-brown, semi-solid	9.7
	chloroform	dark- green, solid	15.1
	methanol	dark-brown, solid	48.4

Isolation of Emodin (I)

The crude chloroform extract (12.0 g) was subjected to silica gel column chromatography using gradient solvent mixtures of 100% petroleum ether, petroleum ether/chloroform (1:1), 100% chloroform and chloroform/methanol (5:2) to give 15 fractions. Fractions 9 and 12 which gave the same R_F values (0.35, chloroform:methanol, 3:1) on TLC were combined together and resubjected to column chromatography with chloroform and methanol, (1:1) to give emodin (I) as reddish orange crystals (42.3 mg) with melting point 258-260°C (Gow-chin *et al.* 1998, 259-260°C).

Isolation of Sucrose (II)

The methanol extract (48.4 g) was redissolved in 95% aqueous methanol (500 ml) and then extracted successively with n-hexane, chloroform, ethyl acetate and n-butanol. The n-butanol extract was chromatographed on silica gel column chromatography with chloroform:methanol gradients of increasing polarities followed by rechromotography of fractions 5-8 to yield 11 fractions. Fraction 5 afforded yellow solid material which was washed with methanol to give yellowish crystals and reextracted with absolute ethanol to give sucrose (37.8 mg) with melting point 193-194°C (Pouchert and Camphell 1974, 190-192°C) and R_F value 0.37 (100% ethyl acetate).

Isolation of Morin (III)

Fractions 24-29 from the above column were combined and rechromatographed to yield a yellow powder. Recrystallisation from acetone gave morin as pale yellow needles (55.6 mg) with melting point 303-304°C (David and Milne 1995, 303.5°C).

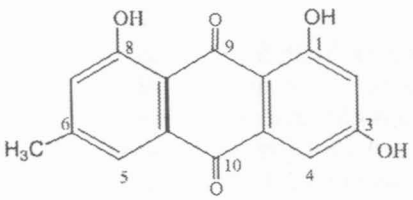
Isolation of Rutin (IV)

Fractions 33-36 obtained from the above column were also combined and resubjected to column chromatography eluted with 100% chloroform with increasing amounts of methanol to give 14 fractions. Fractions 8-10 gave a yellow solid material which was recrystallised in acetone to yield rutin (IV) as yellow crystals (38.5 mg) with melting point 212-214 (C (Hill *et al.* 1991, 214-215 (C).

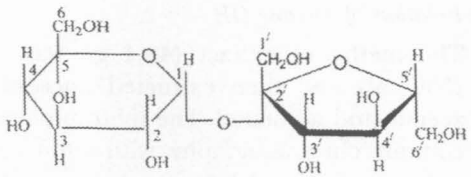
Antimicrobial Assay

The microorganisms were obtained from the culture collection of the Department of Biotechnology, Universiti Putra Malaysia. 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 (Bergeys 1957). The bacterial strains used were *Bacillus cereus* NRRLUI-1447 and *Pseudomonas aeruginosa* UI-60690 while the antifungal strains were *Aspergillus ochraceus* NRRL 398, *Candida lipolytica* ATCC 2075, *Sacchromyces cereviseae* NRRL 2034 and *Sacchromyces lipolytica.*

Antimicrobial activity of the isolated compounds were tested using the disc diffusion method according to Bauer *et al.* (1966). The discs were prepared by impregnating them in an 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 standard antifungal and antibacterial agents respectively. 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.



(I)



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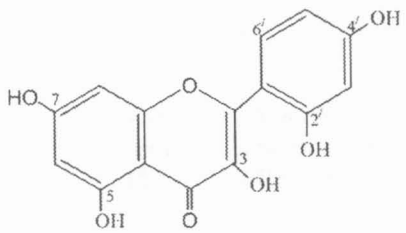

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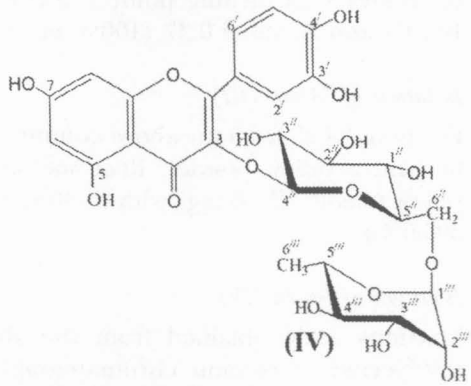

(IV)

RESULTS AND DISCUSSION

Four compounds have been successfully isolated from *Ficus elastica* by column chromatography. These compounds have been identified to be emodin (I), sucrose (II), morin (III) and rutin (IV).

There were four protons in the aromatic region of the ^{1}H-NMR spectrum appearing as singlets at δ 7.56, 7.30, 7.05 and 6.65 and assigned for proton at C-4, C-5, C-2 and C-7 respectively. The two sharp singlets in the downfield region at δ 12.25 and 12.18 were assigned to the two chelated hydroxyl groups at positions 1 and 8. A small broad singlet at δ 10.62 indicated the presence of the free hydroxyl group at C-3, whereas the singlet at δ 2.44, integrated for three protons, was assigned to a methyl substituent.

The ^{13}C-NMR spectrum indicated the presence of fifteen carbons in the structure. The two carbonyl carbons occurred at very low fields, 189.8 and 181.5 ppm. The high field signal at 21.5 ppm is due to the methyl group at C-6. The mass spectrum gave a molecular ion peak at m/z 270 which corresponded to the molecular formula $C_{15}H_{10}O_5$. On comparison, the spectral with published data (Suraj 1989; Parkas and Anderson 1982) suggested the structure to be 1, 3, 8-trihydroxy-6-methyl-9, 10-anthracenedione (emodin).

Compound II was identified as sucrose based on IR, ^{1}H, ^{13}C-NMR, MS and on comparison with published data.

The aromatic region of the ^{1}H-NMR spectrum of compound III showed the presence of five aromatic protons. A pair of doublets (J=1.7 H_z) occurred at δ 8.06 and 6.92. These were assigned to the *meta* protons at H-6 and H-8, respectively. Another doublet observed at δ 7.26 with coupling constant 2.0 Hz was assigned for proton at H-3′. The proton at H-6′ occurred as doublet due to coupling with proton at H-5′ which in term coupled to H-3′. The three singlets occurring at very low field regions at δ 12.62, 10.79 and 9.85 (×2) could be assigned to the four hydroxyl groups at C-5, C-7 and C-2′ and C-4′ (overlapped) respectively. The ^{13}C-NMR spectrum assignments were based on a DEPT experiment. The DEPT spectrum also showed the presence of fifteen carbon atoms consisting of ten quartenary carbons (C-7, C-9, C-4′, C-2, C-2′, C-5, C-4, C-3, C-1′ and C-10) and five methine carbons (C-6, C-8, C-3′, C-5′ and C-6′). The mass spectrum showed the presence of a molecular ion at m/z 302 corresponding to the molecular formula $C_{15}H_{10}O_7$ in agreement with a flavonol skeleton (Markham 1982) and structure of morin (i.e., 3, 5, 7, 2′, 4′ -pentahydroxyflavone).

The UV spectrum of compound IV showed absorptions at λ_{max}360.0 and 265.0 nm, indicating the presence of 3-O-substituted flavonol skeleton (Geissan, 1962). The ^{1}H-NMR spectrum clearly establishes the presence of chelated hydroxyl groups with the occurrence of a sharp singlet at δ 12.61 for 5-OH. In addition, the ^{1}H-NMR spectrum also showed the presence of five protons which appeared as doublet at δ 7.56 (J = 2.2 H_z), 6.81 (J = 2.2 H_z), 6.39 (J = 2.2 H_z) and 6.18 (J = 2.2 H_z) and were assigned to H-2′ and H-6′ (overlapped), H-5′, H-8 and H-6 respectively. H-1″ for glucosyl was observed as doublet at δ 5.33. The one singlet peak at δ 4.36 revealed the presence of one proton at H-1‴ for rhamnosyl, in which the methyl group of rhamnosyl appeared at δ 1.02.

The carbon assignments were made by DEPT experiment. The spectrum showed the presence of twenty-seven carbons consisting of ten quartenary carbons (C-4, C-7, C-9, C-5, C-2, C-10, C-4′, C-3′, C-3 and C-1′), fifteen methine carbons (C-6′, C-5′, C-2′, C-6, C-8, C-1‴, C-1″, C-3″, C-5″, C-2″, C-4‴, C-4″, C-3‴, C-2‴ and C-5‴), one methyl carbon at C-6‴ and one methylene carbon at C-6″. The mass spectrum showed the presence of a molecular ion at m/z 610 corresponding to the molecular formula $C_{27}H_{30}O_{16}$. The structure of this compound was thus established as 5, 7, 3′, 4′-tetrahydroxy 3-O-rahmanoglucosyl (rutin).

The four compounds isolated showed antibacterial activity towards the *Bacillus cereus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). The activity ranged between weak, moderate and strong based on the diameter of inhibition zones. However, no antifungal activity was observed against the four species of fungi (Table 2).

TABLE 2
Antimicrobial activity of compounds isolated (concentration 100 μg/ml, methanol) from *Ficus elastica*

compound	bacteria		fungi			
	B. cereus	*P. aeruginosa*	*A. ochraceous*	*C. lipolytica*	*S. areviseae*	*S. lipolytica*
emodin	+ +	+ +	-	-	-	-
sucrose	+	+	-	-	-	-
morin	+ + +	+ + +	-	-	-	-
rutin	+ + +	+ + +	-	-	-	-

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)

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