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Alkaloids from *Aegle marmelos* (Rutaceae)

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Abstract. Two alkaloids, N-2-hydroxy-2-(4-methoxyphenyl)-ethylcinnamamide (aegeline), and 4,7,8-trimethoxy-furoquinoline (skimmianine) were isolated from *Aegle marmelos*. The structure of the compounds were confirmed by spectroscopic analysis and by comparison with the data reported previously. The bioassays of the isolated compounds against some microbes and cancer cell lines were also carried out.

Abstrak. Dua alkaloid, N-2-hidroksi-2-(4-metoksifenil)-etilsinnamamida (aegeline), dan 4,7,8-trimetoksi-furokinolin (skimmianine) telah dipencilkan daripada *Aegle marmelos*. Struktur sebatian dikenalpasti dengan teknik spektroskopi dan dibuat perbandingan dengan data yang telah dilaporkan. Biocerakian daripada dua alkaloid tersebut terhadap mikrob uji dan sel kaser telah juga dilakukan.

Keywords : *Aegle marmelos*, Rutaceae, alkaloids, antimicrobial activity, cytotoxic test

Introduction

Aegle marmelos is a tree belongs to Rutaceae family, which grows in India, Bangladesh and Southeast Asia. This plant is used in traditional medicine treatments, such as for intermitent fever, intestinal ailments, fertility control, treatment after childbirth and fish poison [1].

Previous studies revealed that some compounds including cinnamic acid and coumarins derivates and alkaloids have been isolated from *Aegle marmelos* [1,2,3]. In this study aegeline (**1**) was isolated from the leaves, whereas skimmianine (**2**) was obtained from the roots of the plant. The biological activity of the isolated compounds against some microbes and its cytotoxicity against CEM-SS (T-cell lymphoblastic leukemia) cells test was evaluated and the IC_{50} values were calculated.

Experimental

General

Melting points (uncorrected) were determinated on Kohfler melting points apparatus. The IR spectra were recorded using KBr disc on Perkin Elmer FTIR spectrophotometer model 1650. ^{1}H and ^{13}C NMR spectra were obtained on JEOL spectrometer at 500 and 125 MHz, respectively with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on an AE1-MS 12 spectrometer. The column chromatography was carried on silica gel (Merck 9385), and Merck silica gel 60 PF254 was used for analytical TLC analysis.

Plant Material

The plant in this study was collected from Yogyakarta, Indonesia in 1998. The plant was identified by by Dr.Suwijio Pramono of Department of Biological Pharmacy, Faculty of Pharmacy, Gadjah Mada University and the voucher specimen (SP 1098) was deposited at the herbarium of the institution.

Isolation of the compounds

Part of the plant (leaves, roots) were air dried and ground. The powdered samples were successively extracted with petroleum ether, chloroform and methanol. The crude extracts were subjected to the flash column chromatography and followed by mini column separation.

Aegeline (1) : IR (cm^{-1},KBr disc) ν_{max} : 3375, 3266, 3092, 2935, 1653, 1606, 1566, 1514, 1437, 1402, 1244, 1113, 1076,1038. Mass spectrum *m/z* (% intensity) : 297(M^+,10), 279(100), 223(14), 207(30), 161(100), 131(90), 103(50), 77(53).

Skimmianine (2) : IR(cm^{-1},KBr disc) ν_{max} : 3118, 3069, 2979, 2945, 2839, 1621, 1579. Mass spectrum *m/z* (% intensity) : 259 (M^+, 59), 244 (100), 230 (51), 213 (25), 201 (26), 184 (5), 173 (12), 156 (5),

144 (6), 130 (12). ^{1}H NMR (500 MHz, CDCl3) δ: 7.96(*d*,*J*=9.5,1H,H-5), 7.53(*d*,*J*=2.5,1H,H-2), 7.19 (*d*,*J*=9.5,1H,H-6), 6.97(*d*. *J*=2.5,1H,H-3), 4.38(*s*,3H,OMe-C-4), 4.05(*s*,3H,OMe-C-7), 4.12(*s*,3H,OMe-C-8). ^{13}C NMR (125 MHz, CDCl3) δ : 164.3(C-10), 157,1(C-4), 152.1(C-13), 142.9(C-2), 141.9(C-7), 141.4(C-8), 118.2(C-5), 114.8(C-11), 111.9(C-6), 104.6(C-3), 101.9(C-10), 61.6(C-8), 58.9(C-4), 56.8(C-7).

Bioassay

Antimicrobial activity was determinated by diffusion method. The compounds were impregnated on the paper disks and setted in petri disks where the microbes were innoculated in the agarose media. The microbes used was *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Candida lipolytica, Aspergillus ochraceous, Saccharomyces cerevisiae.* Cytotoxicity test was determined against T-cell lymphoblastic leukemia. The IC_{50} of the compounds were measured based on the amount of enzyme that was released by cancer cell.

Results and Discussion

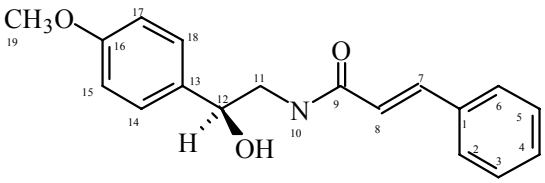
Compound (**1**) appeared as white crystals, m.p. at 179-180^{o}C, lit.[4] m.p.173-175^{o}C, was isolated from petroleum ether extract of *Aegle marmelos* leaves. The UV spectrum showed bands at λ_{max} 219, 222 and 274 nm, which was typical of a *trans*-cinnamide group. IR spectrum showed the presence of N-H as sharp peaks around 3375 cm^{-1}, while broad peak at 3266 cm^{-1} exhibited OH group. Peaks at 3092 and 3010 cm^{-1} revealed the aromatic C-H, whereas peak at 2935 cm^{-1} was due to aliphatic C-H stretching vibration. The existence of carbonyl group conjugated to a double bond was represented by the strong peak at 1653 cm^{-1} suggesting that α,β unsaturated ketone exist in *trans* conformation. In addition, peak at 1606, 1566 cm^{-1} were attributed of C=C and aromatic ring system, while 1244 cm^{-1} was the absorption of N-H stretching. The presence of C-O was shown by peak at 1038cm^{-1}. Mass spectrum showed molecular ion at *m/z* 297 that corresponds to molecular formula $C_{18}H_{19}N0_3$.

Table 1: The assignment of ^{1}H and ^{13}C NMR signals (in $CDCl_3$) of aegeline (**1**)

C position	δ^{1}H (*J*,Hz)	δ ^{13}C	COSY correlation	HMQC correlation	HMBC correlation
1	-	133.9	-	-	H_2, H_6,H_7
2	7.53 (*dd, J=6.7, 2.5*)	127.5	-	H_2, H_6	H_3, H_5, H_6
3	7.38 (*d*,*J*=6.7)	128.5	-	H_3, H_5	H_2, H_6
4	7.41 (*s*)	129.5	-	H_4	H_2, H_6
5	7.38 (*d*,*J*=6,7)	128,5	-	H_3, H_5	H_2, H_6
6	7.52 (*dd.J=6.7, 2.5*)	127.5	-	H_2, H_6	H_2,H_3,H_5,
7	6.51 (*d*,*J*=15.6)	120.1	H_8	H_7	H_8
8	7.59 (*d*,*J*=15.6)	140.9	H_7	H_8	H_7
9	-	167.3	-	-	H_7, H_8
10	4.13 (*d*,*J*=4.3)	-	-	-	-
11	3.42 (*m*)	48.5	H_{12}	H_{11}	H_{10}
12	4.79 (*dd, J*=8.2, 3.9)	72.2	H_{11}	H_{12}	H_{11}
13	-	158.9	-	-	H_{14},H_{18}
14	6.90 (*d*,*J*=8.9)	126.8	-	H_{14}	H_{15}, H_{17}, H_{18},
15	7.33 (*d*,*J*=8.9)	113.5	H_{14}	H_{15}, H_{17}	H_{14},H_{18}
16	-	134.5	-	-	H_{15}, H_{17}, H_{19}
17	7.33 (*d*,*J*=8.9)	113.5	H_{18}	H_{15}.H_{17}	H_{14}, H_{18}
18	6.90 (*d*,*J*=8.9)	126.8	H_{17}	H_{18}	H_{14}, H_{15}, H_{17}
19	3.81 (*s*)	54.9	-	H_{19}	-

^{1}H-NMR spectrum exhibited that a singlet at δ 3.81, integrated for three protons was attributed a methoxy group (H-19). The presence of a pair of doublet at δ 7.59 and δ 6.52 (both with *J*=15.6 Hz) represented *trans* olefinic protons of H-8 and H-7, respectively. Mono-substituted aromatic protons were represented by H-2 and H-6 which appear as doublet of doublet at δ 7.53 (*J*=6.7, *J*=2.5 Hz), while H-3 and H-5 resonate as doublet at δ 7.38 (*J*= 6.7 Hz). Another pair of doublet (*J*=8.8Hz) were given by H-15 and H-17 (δ 7.33) which ortho-coupled to H-14 and H-18 (δ 6.90). The inequivalent methylene protons at C-11 appeared as two doublet of doublet at at δ 3.67 (*dd, J*=13.8, *J*=3.4 Hz) and δ 3.43 (*dd, J*=3.4, *J*=8.5 Hz). The neighbouring methine proton at C-12 which is coupled to protons (H-11) resonates to give another doublet of doublet at

δ 4.80 (*dd, J*=8.5, *J*=3.4 Hz). All of the couplings interaction between protons were confirmed by COSY spectrum , such as the crosspeaks which were due to the interaction between H-7 and H-8 and between appropriate aromatic protons (Table 1). ^{13}C-NMR spectrum gave absorption peaks representing mostly aromatic, olefinic and sp^3 carbons. The presence of carbonyl group was shown by peak at δ 167.3. The assignment of ^{1}H and ^{13}C-NMR data was further substantiated by HMQC and HMBC spectrum, which the complete assignment and correlations are summarized in Table 1. Based on these spectral data and comparison with the previous report [3] the compound (**1**) was identified as of *N*-2-hydroxy-2(4-methoxyphenyl) ethyl-cinnamamide (aegeline).

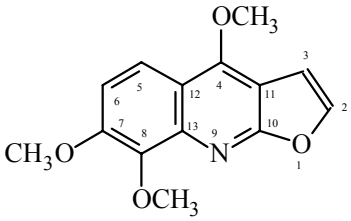


Aegeline (**1**)

Compound (**2**) was obtained from the roots, appeared as yellowish white crystals, melts at 180-182^{o}C, Lit.[5] m.p.178^{o}C. UV spectrum shows λ_{max} MeOH 332 nm (logε=4.02), 248 nm (logε=5.33), due to the presence of furan and benzen ring system. IR spectrum exhibits the presence of the C-H aromatic and C-H aliphatic at 3102 and 2945 cm^{-1}, respectively. Peaks at 1621, 1500 and 1383 cm^{-1} appeared due to C=C or aromatic ring system, while C-O bond was represented by peak at 1271 and 1096 cm^{-1}. However, the spectrum did not show any absorption due to N-H or C=O groups. Mass spectrum showed molecular ion at odd number *m/z* 259, corresponds to molecular formula $C_{14}H_{13}NO_4$. The presence of fragment ions at *m/z* 230, 244 and *m/z* 216 indicated substitution at C-4 and C-8 of the molecule [6].

^{1}H-NMR spectrum showed three sharp singlets at δ 4.38, 4.50 and 4.08, assigned to three OCH_3 groups. The rest of the signals occurred in the aromatic region. A pair of doublet at δ 7.96 and δ 7.10 with coupling constant (*J*=9.5 Hz) was assigned to *orto* coupling protons at C-5 and C-6 [6]. Another pair of doublet at δ 7.53 and δ 6.97 (*J*=2.5 Hz) was typical of adjacent of furan protons (H-2 and H-3) [7]. ^{13}C NMR spectrum showed the presence of fourteen carbons in which three of them were methyl carbons resonated at 56.8, 58.9 and 61.6 ppm. Other signals included four methine and seven quaternary carbon absorptions.

Based on its similarity with the previous report [5], the structure of the compound (**2**) was suggested as 4,7,8-trimethoxyfuro[2,3-b]quinoline or skimmianine previously isolated from *Haplophylum tuberculatum* (Rutaceae) [6]. Both alkaloids isolated from the plant sample did not show any activity against all the pathogenic microbes used in the test. However, aegeline exhibited weak cytotoxic activity against cancer cell lines with IC_{50} value 22.5 μg/ml, whereas skimmianine was inactive.



Skimmianine (**2**)

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