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(R)-N-trans-feruloyloctopamine from the root timber of *Melochia umbellata* (Houtt.) Stapf var. *Visenia* (Paliasa)

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Abstract. A phenolic amide namely (R)-N-trans-feruloyloctopamine has been isolated from chloroform fraction of the root timber *Melochia umbellata* (Houtt.) Stapf var. *Visenia* (Paliasa). Isolation compound has been done by maceration, fractionation, and purification. The molecular structure was determined by IR spectroscopy, NMR 1D and 2D (¹H-NMR, ¹³C-NMR, HSQC and HMBC). Isolate formed yellow paste (83.4 mg) with melting point of 156 – 158 °C. The λ_{maks} data on the UV-Vis spectrum of 333.80 nm (3.795) indicate compound has a long conjugated double bond. Data IR ν_{max} cm⁻¹ for groups as follows : 3327 (-OH), 2933 (C-H aliphatic), 1653 (C=O amide), 1593 (olefin). The data of NMR 1,2D show that this compound contains 18 carbon atoms, consisting of: 1 methyl, 1 methylene, 11 metin and 5 carbon quartener. Metin carbon consists of 2 alkene protons (olefins), 7 aromatic protons, and 1 alkyl alcohol (δ_{c} 72.58 ppm). NMR data also shows that one quartener carbon as C = O amide group (δ_{c} 166.92 ppm) and one methoxy (δ_{c} 55.35 ppm). The (R)-N-trans-feruloyloctopamine is the first compound found in the genus *Melochia*.

1. Introduction

Paliasa is the term medicinal plant on Sulawesi Island which consist of two different plant species namely *Kleinhovia hospita* L. and *Melochia umbellata* (Houtt.) Stapf. *Melochia umbellata* (Houtt.) Stapf consists of two varieties, namely *M. umbellata* (Houtt.) Stapf var. *Degrabrata* K. and *M. umbellata* (Houtt.) Stapf var. *Visenia*. *M. umbellata* with the name synonym of *Melochia indica* (Gmel.) Kurz. is the native plants in the India to the Philippines, the New Guinea, and the Pasifik Islands [1]. Paliasa, including *M. umbellata* (Houtt.) Stapf var. *Visenia* has been used traditionally in the Province of South Sulawesi (Indonesia) to treat liver and other diseases [2].

Paliasa contains chemical compounds such as steroids, terpenoids, alkaloids and flavonoids. The stem bark of *K. hospita* L. contains 7-hydroxy-6-methoxy-coumarin (scopoletin) and β -sitosterol compounds [3], and two triterpenoid compounds, namely 2,3-dihydroxy-12-oleanan-28-oat and 2-hydroxy-12-oleanan-28-oat from the stem and root bark of *K. hospital* L. [4]. Hexane extract from the root timber of *M. umbellata* (Houtt.) Stapf var. *Degrabrata* found β -sitosterol compound [5], two steroid group namely stigmast-5,22-dien-3-O- β -D-glucopiranoside [6] and β -sitosterol [5], one



compound from triterpenoids group namely 3-acetyl-12-oleanen-28-oic acid [7], phenol compound methyl β - (p-hydroxyphenyl) acrylic [8], the flavonoids compound from the isoflavone group namely 6,6'-dimetoksi-4,4'-dihydroxy-3, 2'-furano-isoflavan [9], melosinone alkaloid compound i.e. Walterion C and the Cleomiscosin A from the coumarin group [10].

Many bioactivity tests to both extracts and pure isolates have been reported. The compounds of stigmast-5,22-dien-3O- β -D-glukopiranoside active against the fungus of *Aspergillus niger* [6], and 3-acetyl 12-oleanan-28-oic-acid active against the fungus of *Bacillus subtilis* and *Candida albicans* [7]. The compounds of 6,6'-dimethoxy-4,4'-dihydroxy-3 ', 2'-furano-isoflavan [9], methyl b-(p-hydroxyphenyl) acrylic [3], and walterion C shows anticancer activity [10]. The leaf extract of *K. hospita* and *M. umbellata* var. Degrabrata can positively protect liver inflammation [11] and also paliasa leaves extract still save in every treatment dosage [12], antioxidant activity and moderate cytotoxic activity against HepG2 liver cancer cells [13].

Recently, a phenolic amide namely (R)-*N-trans*-feruloyloctopamine, has been isolated from chloroform fraction of the bark *M. umbellata* (Houtt) Stapf var. *Visenia* (Paliasa). The molecular structure was determined by IR spectroscopy, NMR 1D and 2D ($^1\text{H-NMR}$, $^{13}\text{C-NMR}$, HMQC and HMBC). The (R)-*N-trans*-feruloyloctopamine is the first compound found in the genus *Melochia*, even though to be first discovered in the Malvaceae family.

2. Methods

2.1. Time and location

The research was conducted in February 2018 to March 2019 at the Organic Chemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences of Hasanuddin University in Makassar and Bandung Institute of Technology.

2.2. Materials and Equipment

Materials used in this study are a sample the root timber of *M. umbellata* (Houtt) Stapf var. *Visenia*, Organic solvent used quality p.a and technical; *n*-hexane, chloroform, ethyl acetic, acetone and methanol, silica gel Merck (7730, 7733 and 7734), and cerium sulphate solution 2 %.

Equipment used in this study were glass tools commonly used in the laboratory, vacuum liquid chromatography equipment, press column chromatography, gravity column chromatography, thin layer chromatography (TLC), chromatotron, a developer room (chamber), micropipette, microplate, antimicrobial test equipment, ultraviolet light (λ , 254 and 360 nm), evaporator tools Buchi, melting point apparatus Fisher Johns, UV Vis, FTIR 8501 Shimadzu and NMR Agilent 500 MHz.

2.3. Isolation and Spectroscopic Analysis

Extraction is done by total maceration method. First, the root timber powder *M. umbellata* (Houtt.) Stapf var. *Visenia* was macerated using methanol, then partitioned liquid-liquid using a solvent: *n*-hexane, chloroform, ethyl acetate, and methanol. Each filtrate obtained was then evaporated until is obtained viscous extract of each fraction, namely: fraction *n*-hexane, ethyl acetate and methanol fraction. Each fraction obtained is then weighed to find out its weight. Purification was carried out using chromatographic techniques such as vacuum liquid chromatography, press column chromatography, gravity column chromatography, and chromatotron with the appropriate eluents. The purity test was performed using thin layer chromatography analysis and melting point measurements.

The molecular structure was determined by IR spectroscopy, NMR 1D and 2D ($^1\text{H-NMR}$, $^{13}\text{C-NMR}$, HSQC and HMBC).

3. Results and Discussion

3.1. Isolation

The plants bark powder of *M. umbellata* var. *Visenia* plants (10 kg) was extracted by maceration with methanol 1 x 24 hours (3 times). The methanol extract was concentrated by a low-pressure rotary evaporator and methanol extract (116,26 g) was obtained. The methanol extract was partitioned with

of liquid - liquid extraction using solvents with increasing polarity; *n*-hexane, chloroform and ethyl acetate. At the root timber obtained *n*-hexane extract as much as 10.24 g, chloroform extract as much as 41.28 g, and ethyl acetate as much as 11,1 g.

Chloroform extract as much as 30.78 grams was fractionated using vacuum column chromatography (KKV) method using *n*-hexane (100%), ethyl acetate: *n*-hexane in gradually from a ratio of 1: 9 to 9: 1, ethyl acetate (100%). The initial fractionation results obtained 54 main fractions, then fractions that showed the same profile spot on the TLC plate were combined, resulting in 12 combined fractions (A-K) (Figure 1).

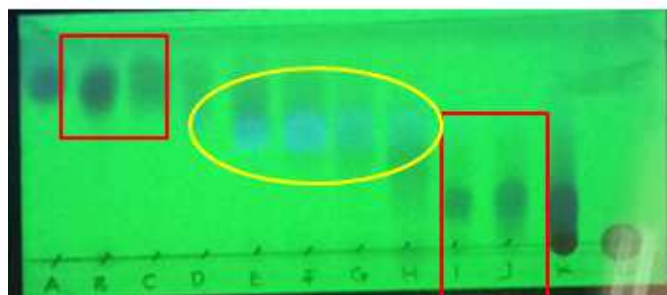


Figure 1. KKV profile spot of chloroform extract

As much as 1.5 grams of the IJ fraction done KVC uses eluent: *n*-hexane : ethyl acetate in the ratio of 4: 6; 3.5: 6.5; 3: 7, and 4: 6, produce 22 subfractions, then combined based on the similarity of R_f to 11 combined subfractions (IJ1-IJ10). Subfraction of IJ7a and IJ7b respectively in chromatotron with eluent chloroform: methanol in the ratio of 19: 1 and 9: 1. Subfraction of IJ7a produced 31 sub-subfractions, where sub-subfraction 18 to sub-subfraction 24 formed yellow paste (83.4 mg) with melting point of 156 – 158 °C was pure compound, shown in Figure 2.

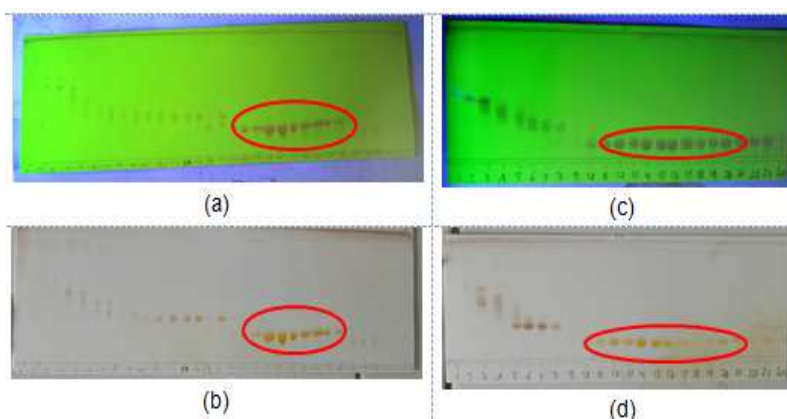


Figure 2. Spot profile of isolated compound of (R)-*N*-*trans*-feruloyloctopamine

3.2. Spectroscopic Data Analysis

The maximum wavelength (λ_{maks}) data on the UV-Vis spectrum of 333.80 nm with absorbance of 3.795 indicate that this compound has a long conjugated double bond.

The IR spectrum in Figure 3 shows a wide absorption band at wave number 3327.21 cm^{-1} for OH, a strong and sharp absorption band at 1653.00 cm^{-1} for a carbonyl amide (C=O amide) and 1031.92 cm^{-1} for a carbon-oxygen single bond (C-O alcohol). The at wave number 2845.00 cm^{-1} and 2933,73 cm^{-1} for C-H bond of aliphatic structure which are reinforced by absorption band at 1450.47

cm^{-1} for C-H bending of the CH_2 group. The weak absorption band between 3000 and 3100 cm^{-1} for C-H is not saturated, and the presence of absorption bands at 1595.13 and 1514.12 cm^{-1} shows that the unsaturated group is an aromatic group. Furthermore, the absorption band at 837.11 cm^{-1} shows that the aromatic group is para substituted. The presence of absorption bands above 1600 cm^{-1} as the shoulder of the absorption band at 1595.13 cm^{-1} shows the presence of olefins, and the absorption band at 975.98 cm^{-1} states that the olefins is trans substituted.

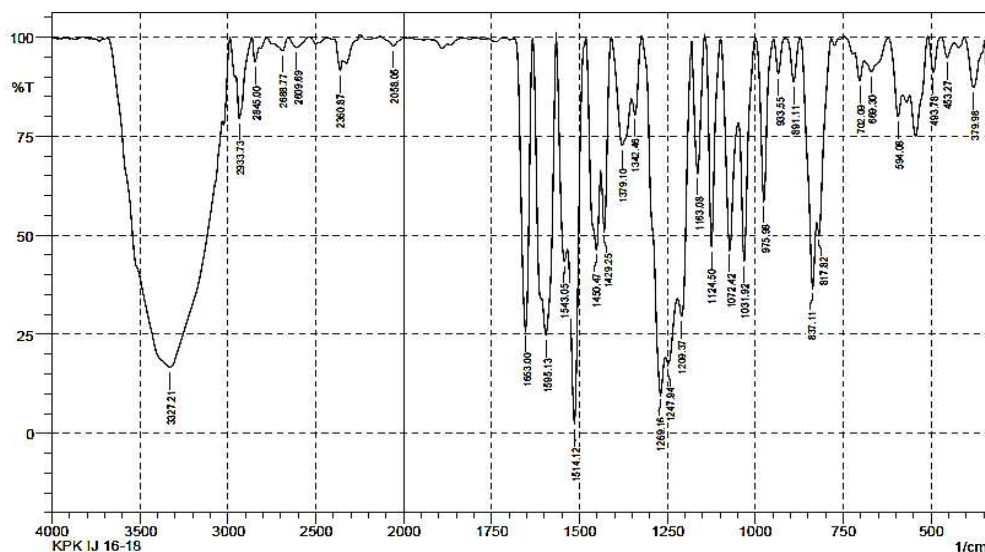


Figure 3. FTIR spectrum of (R)-N-trans-feruloyloctopamine

The $^1\text{H-NMR}$ spectrum shows the presence of signal at chemical shift (δ_{H}) 7.18 ppm (H-2), δ_{H} 6.85 ppm (H-5), δ_{H} 7.06 ppm (H-6), δ_{H} 7.52 ppm (H-7), δ_{H} 6.63 ppm (H-8), δ_{H} 4.79 ppm (H-7'), δ_{H} 3.42 ppm (Ha-8') and 3.65 ppm (Hb-8'), δ_{H} 3.86 ppm (H-9), and two symmetrical signals at δ_{H} 7.25 ppm (H-2'/6') and 6.82 ppm (H-3'/5').

The $^{13}\text{C-NMR}$ spectrum shows the presence of signal at chemical shift (δ_{C}) 127,20 ppm (C-1), 110,47 ppm (C-2), 147,76 ppm (C-3), 148,41 ppm (C-4), 115,29 ppm (C-5), 121,89 ppm (C-6), 140,27 ppm (C-7), 118,60 ppm (C-8), 166,92 ppm (C-9), 134,13 ppm (C-1'), 127,18 (C-2'/6'), 114,95 ppm (C-3'/5'), 156,70 ppm (C-4'), 72,58 ppm (C-7'), 47,81 ppm (C-8'), and 55,35 ppm (C-9'). The total is 18 carbon atoms, consist of 1 methyl (CH_3), 1 methylene (CH_2) 11 metin (CH), and 5 quaternary carbon.

Based on the data $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$, can be described that the proton at δ_{H} 6.86 ppm (H-5) which is bonded to the carbon at δ_{C} 115.29 (C-5), and proton at δ_{H} 7.06 ppm (H-6) which is attached to the carbon at δ_{C} 121.89 ppm (C-6) in the aromatic B ring has the same coupling constant of 8.0 Hz indicating that the two protons are coupling ortho to each other. The $^1\text{H-NMR}$ spectrum also showed two doublet signal integration 2 (symmetric) ie proton at δ_{H} 7.25 ppm (H-2'/6') are attached to the carbon δ_{C} 127.18 ppm (C-2'/6') and the proton at δ_{H} 6.82 ppm (H-3'/5') which is bound to the carbon δ_{C} 114.95 ppm (C-3'/5') in the aromatic A ring has the same coupling constant of 8.5 Hz indicating that the two protons are also coupling ortho to each other. In addition, the coupling constant of 15.5 Hz indicates a trans relationship (two trans olefinic) between proton at δ_{H} 7.52 ppm (H-7) and proton at δ_{H} 6.63 ppm (H-8).

The HMBC spectrum shows that the proton at δ_{H} 7.25 ppm (H-2'/6') in the aromatic core of A ring have correlation distance with the carbon at δ_{C} 72.58 ppm (C-7') which are in the acyclic chains and quaternary carbon at δ_{C} 156.70 ppm (C-4') which binds to the hydroxyl group. Proton at δ_{H} 6.82 ppm (H-3'/5') correlated distance with carbon at δ_{C} 134.13 ppm (C-1').

At the B ring, the HMBC spectrum data shows distance correlation of proton at δ_H 7.18 ppm (C-2) to carbon at δ_C 121.89 (C-6), quaternary carbon at δ_C 147.76 ppm (C-3), acyclic chain carbon at δ_C 140.27 (C-7) and carbon at δ_C 148.41 ppm (C-4). Proton at δ_H 7.06 (H-6) correlated distance with carbon at δ_C 110.47 ppm (C-2), carbon at δ_C 140.27 (C-7), and quaternary carbon at δ_C 147.76 ppm (C-3). The proton at δ_H 6.85 ppm (C-5) correlated distance with the quaternary carbon at δ_C 127.20 ppm (C-1), δ_C 147.76 ppm (C-3), and δ_C 148.42 ppm (C4).

Table 1. Interpretation Data HSQC and HMBC of (R)-*N-trans*-feruloyloctopamine

No	HSQC				HMBC
	$^1\text{H-NMR}$ ppm (I,m,J) ^a	$^{13}\text{C-NMR}$ Ppm (Gugus) ^a	$^1\text{H-NMR}$ ppm (I,m,J) ^b	$^{13}\text{C-NMR}$ (ppm) ^b	
1	-	127,20 (CH)	-	127,08	-
2	7,18 (1H,d, 1,5)	110,47 (CH)	7,12 (1H, brs)	110,41	C-6, C-7, C-3, C-4
3	-	147,76 (C)	-	148,13	-
4	-	148,41 (C)	-	148,75	-
5	6,85 (1H,d, 8)	115,29 (CH)	6,79 (d, 8)	115,31	C-1, C-3, C-4
6	7,06 (1H,dd, 8 dan 8)	121,89 (CH)	7,02 (dd; 8,2)	122,13	C-2, C-7, C-3
7	7,52 (1H,d, 15,5)	140,27 (CH)	7,44 (d;15,5)	141,09	C-2, C-6, C-9
8	6,63 (1H,d, 15,5)	118,60 (CH)	6,46 (d; 15,5)	117,46	C-1, C-9
9	-	166,92 (C)	-	168,34	-
1'	-	134,13 (C)	-	133,56	-
2'/6'	7,25 (1H,d, 8,5)	127,18 (CH)	7,22 (d; 8,5)	127,29	C-7', C-2'/6', C-4'
3'/5'	6,82 (1H,d, 8,5)	114,95 (CH)	6,77 (d; 8,5)	114,96	C-1', C-3'/5'
4'	-	156,70 (C)	-	156,91	-
7'	4,79 (1H,qi, 4,0)	72,58 (CH)	4,73 (dd; 7,5;5,0)	72,22	C-8', C-2'/6'
8'	3,42 (1H, brs) 3,65 (1H, brs)	47,81 (CH ₂)	3,44 (dd;13,5) 3,54 (dd; 13,5)	47,50	C-7', C-9
9'	3,86 (1H,s)	55,35 (CH ₃)	Td	55,22	C-3

^a Isolated compound ($^1\text{H-NMR}$, 500 MHz, acetone-d₆; $^{13}\text{C-NMR}$, 125 MHz, acetone-d₆)

^b Dong *et al* 2004 ($^1\text{H-NMR}$, 500 MHz, CD₃OD; $^{13}\text{C-NMR}$, 125 MHz, CD₃OD)

Then in the acyclic chain, proton at δ_H 7.52 ppm (C-7) correlated distance with carbon at δ_C 121.89 ppm (C-6) at the aromatic core of B ring, and carbon atom of carbonyl amide at δ_C 166.92 ppm (C-9). A methoxy group at δ_H 3.86 ppm (H-9') which is bound to carbon at δ_C 52.22 ppm (C-9') correlated distance with carbon at δ_C 147.76 ppm (C-3) at the aromatic core of B ring.

Based on the data above, this compound has a similarity in position, chemical shift, and coupling constants of previously found compounds namely (R) -*N-trans*-feruloyloctopamine [14,15].

The interpretation data of HSQC and HMBC is presented in Table 1, and the molecular structure of the isolated compound (*R*)-*N*-*trans*-feruloyloctopamine which has molecular formula $C_{18}H_{19}NO_5$ and chemical name (IUPAC) is 2-propenamide, N-[2-hydroxy-2-(4-hydroxyphenyl) ethyl]-3-(4-hydroxy-3-methoxyphenyl)-,-(2*E*), shown in Figure 4.

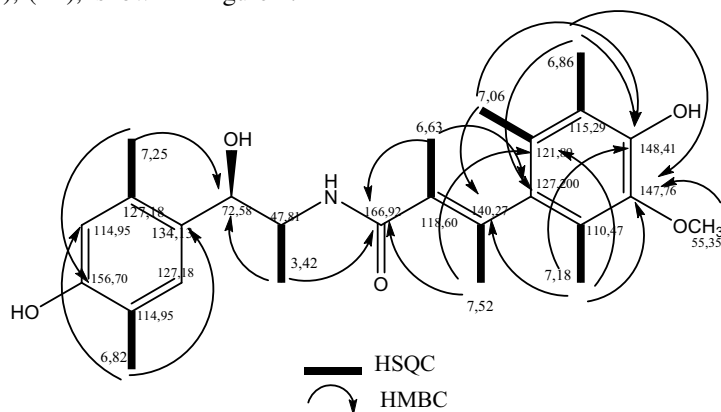


Figure 4. Compounds of (*R*)-*N*-*trans*-feruloyloctopamine

The figure 4 above shows that there are 2 benzene rings, that is disubstitute and trisubstitute benzene. The disubstitute benzene is formed from two symmetrical signals coupled to each other, namely signals at δ_C 127.18 ppm and 114.95 ppm (coupling values equal to 8.5, coupling ortho). HMBC data show that δ_H 7.25 ppm bound to carbon at δ_C 127.18 correlates with quaternary carbon at δ_C 156.70 ppm which binds to one substituent, and δ_H 6.82 ppm bound to δ_C 114.95 ppm correlates with quartener carbon at δ_C 134.13 ppm which also binds to one sustituent. The trisubstitute benzene is formed from aromatic signals at δ_C 115.29 ppm and δ_C 121.89 ppm (coupling value equal to 8.0, ortho-coupled). The HMBC data show that signals at δ_C 121.89 ppm correlate with CH signals aromatic at δ_C 110.47 ppm and δ_C 140.27 ppm. While signals at δ_C 115.29 ppm correlate with 3 quaternary carbon signals (127.20 ppm; 147.76 ppm; 148.42 ppm), which bind substituents, respectively.

4. Conclusion

A phenolic amide namely (*R*)-*N*-*trans*-feruloyloctopamine has been isolated from chloroform fraction of the root timber *Melochia umbellata* (Houtt) Stapf var. *Visenia* (Paliassa) is the first to be found in the genus *Melochia*.

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