IDENTIFICATION OF TRITERPENOIDE COMPOUND FROM  
*Polyscias fruticosa* Harm. (Araliaceae) ROOT BARK

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**ABSTRACT**

Kedondong laut (*Polyscias fruticosa*) is one of medical plants which is useful to increase poultry immune system, antiviral, antibacterial, antidisentry and for curing diuretic and gardiasis. The research purpose is to find out the kind of triterpenoides compound in kedondong laut root bark. In the pre-experiment has indicated positif for Liebermann burchard test for crude extract. It means that reagent showed that there were active compounds of saponin triterpenoides in root extract. Isolation of saponins were carried out based on extraction using methanol followed by dietil eter and n-buthanol. the isolat was then separated by preparative thin layer chromatograpy (TLC) in Silica gel F254 plates using solvent combination of cloroform/methanol/water (20/60/10) and resulting 3 isolates of oleanane and oleanene triterpenoid. Each isolates was analyzed using Spectrophotometric Infrared (IR) and $^1$H-NMR. The isolate with $R_f$ value of 0.55 supposed to be 3-O-$\beta$-D-Glucopyranosil (1→2) $\beta$-D-Glukopiranosil compound, Isolate 2 with $R_f$ value of 0.64 as 3-O-$\beta$-D-rhamnopyranosil (1→2) - $\beta$-D-rhamnopyranosil (1→3) - $\beta$-D-glucopyranosil compound and isolate 3 with $R_f$ value of 0.77 as 3-O-$\beta$-D-glucopyranosil compound.

**Keywords**: Polyscias fruticosa, kedondong laut, triterpenoide.

**INTRODUCTION**

The important triterpenoid compounds have been found intensively from family of Araliaceae, such as cholchisid A-B [1], cussosaponin A-E [2], kalopanax sapotonin A, and pictoside A-B [3], hederasaponin A-D [4]. Their differences were on carbon position of oxidizing, C=C double bond, type and number of glycoside. These compounds showed a variety of significant bioactivities, including diuretic, antibacterial, anti-inflammation [3], antioxidant, and anticancer [5].

In this paper we reported isolation and structural determination of three major triterpenoides from *Polyscias fruticosa* Harm (synonym: *Panax fruticosum* L.). No report has been published toward this plant. Traditionally, *P. fruticosa* Harm has been used as diuretic material and to increase the poultry immunity by local society. It was known as puding (Melayu), kedondong laut (Sunda and Java), kedundung pethedan (madura), bombu (Makasar), keudem rintek (Minahasa), gurabati (Ternate), and dewu papua (Ambon). Our screening study has indicated triterpenoides, tannin and flavonoide groups were composed methanol extract of the root bark [6]. They have activities as anti-inflammation and antioxidant [7].

**EXPERIMENT**

**General experiment procedure.**

Melting point was determined on micro melting point apparatus and uncorrected. UV and IR spectra were measured on spectrophotometer Shimadzu 160-V and Jasco FTIR-5300. $^1$H-NMR spectra was recorded on Hitachi FT$^1$H-NMR R-1900 (in CDCl$_3$), Bruker AV-300 MHz and $^{13}$C-NMR 100 MHz (in pyridine-d$_5$). Vacuum liquid chromatography was carried out using Merck Si gel 60 GF$_254$ and preparative TLC was performed in pre-coated Si gel plates Merck Kieselgel 60 GF$_254$ 20x20 cm).

**Plant material.**

Sample of root bark was received from Balai Materia Medica, Batu and plant was identified by Drs. Djati Batoro in Laboratory of Taxonomy University of Brawijaya.

**Extraction and isolation procedures.**

Dried and milled of root bark of *P. fruticosa* (3 kg) was extracted exhaustively with methanol at room temperature for 24 hours. Evaporated in reduced pressure of extract resulted brown residue (119 g) and suspended in water following extraction using n-hexane. A portion (20 g) of methanol extract was chromatographed by VLC eluted with n-butanol resulted 4 fractions. Major fraction was then separated by preparative TLC using eluent chloroform : methanol : water (2:6:1) and provided compound with $R_f$ 0.55, 0.64 and 0.73.

**Acid hydrolysis.**

25 mg of each sample was hydrolyzed with 5 mL of 2 N HCl in H$_2$O and refluxed for 4 h. The reaction mixture was neutralized with 2 N NaOH and extracted with chloroform. Chloroform layer was saturated with

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nitrogen and analyzed for triterpenoid aglycone using IR, UV and NMR. Meanwhile, for aqueous layer was examined for sugar by comparison its Rf on silica gel TLC with standard glucose, rhamnose and galactose. R_f value for standard in chloroform : methanol : water (17:6:1) were 0.14 (glc), 0.10 (gal), and 0.31 (rha). Rf for sample provides glucose (0.13), and rhamnose (0.30), respectively.

RESULT AND DISCUSSION

Compound 1 has isolated as brown-black gum. IR spectrum showed strong absorption band in 3400 and 1020 which suggested glycoside group and weak band around 1600 indicated unconjugated C=C double bond vibration. Meanwhile, weak absorption band on 1920-1850 and strong in 1400 recorded vibration of methyl, methylene, and methyne groups. These function groups were supported by NMR spectra. The existence of methyl, methylene and methyne groups recorded at 0.937 (d, 18 H), 1.33 (m, 8 H), and 1.538 (m, 18 H). Meanwhile for unconjugated C=C double bond showed at downfield shift 1.862 (t, 12 H).

Absorption for $^{13}$C-NMR spectra of molecule 1 aglycone very closed and supported with triterpenoid olean-12-ene type with C=C double at C12-13 ($\delta$ 123.1 and 143.5) and glycoside attached at C3 which indicated by $\delta$ value at 81.7 for carbon shift by hydroxyl group. 38.7 (C1), 25.1 (C2), 83.7 (C3), 43.5 (C4), 48.2 (C5), 18.2 (C6), 32.7 (C7), 40.1 (C8), 47.0 (C9), 36.8 (C10), 23.8 (C11), 41.9 (C14), 34.4 (C15), 67.9 (C16), 48.2 (C17), 40.5 (C18), 47.7 (C19), 36.1 (C20), 38.8 (C21), 73.1 (C22), 64.7 (C23), 13.6 (C24), 16.2 (C25), 17.0 (C26), 27.4 (C27), 66.0 (C28), 29.9 (C29), and 20.4 (C30). From TLC of glycoside residue by compared with standard glucose could be suggested that olen-12-ene triterpenoide bind glucose with $\beta$-glycoside bond attached.

Isolate Rf 0.64 which proposed as compound 2 has isolated as brown-black crystal. Its IR spectra provided glycoside absorption band at 3400 (strong) and around 1005 (medium, weak) for O-H and C-O stretching vibration. Carbonyl absorption of glycoside ester was come up at 1740 and supported by absorption at 1000 for C-O stretching (splitting with C-O from ether glycoside). Absorption in the left of 3000 (weak) were band for C-H stretching of methyl and methylene, these band was supported by bending methyl and methylene at 1370. Absorption band closed below to carbonyl band was stretching band for cyclic and straight of C=C double bond.

Analysis toward $^{13}$C-NMR spectra for aglycone isolate Rf 0.64 assigned the structure of molecule 2. Absorption or peak at $\delta$ 123.70 and 145.28 have probed the existence of C=C double bond for olefin positioned at C12 and C13. Meanwhile, C3 attached hydroxyl was shifted at 83.45 and unshielded C28 carboxyl carbon was provided at $\delta$ 182.15. Other data recorded were 39.52 (C-1), 26.31 (C-2), 43.90 (C-4), 49.03 (C-5), 18.95 (C-6), 33.80 (C-7), 40.60 (C-8), 48.24 (C-9), 37.73 (C-10), 24.15 (C-11), 43.00 (C-14), 28.85 (C-15), 24.67 (C-16), 47.70 (C-17), 42.80 (C-18), 47.35 (C-19), 31.60 (C-20), 43.00 (C-21), 33.50 (C-22), 65.02 (C-23), 13.51 (C-24), 16.49 (C-25), 17.87 (C-26), 26.57 (C-27), 33.63 (C-29), and 24.07 (C-30). This structure was identical which was reported by Mshvildadze et al. (2001) as colchysid A-aglycone or oleanolic acid aglycone isolated from Hedera colchica K. KOCH (Araliaceae).
Table 3. Integration of $^1$H-NMR spectra molecule 3 aglycone$^a$

<table>
<thead>
<tr>
<th>Signal</th>
<th>$\delta$ (ppm)</th>
<th>Integration</th>
<th>Splitting</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0.888</td>
<td>9</td>
<td>Multiplet</td>
</tr>
<tr>
<td>b</td>
<td>1.259</td>
<td>14</td>
<td>Singlet</td>
</tr>
<tr>
<td>c</td>
<td>1.601</td>
<td>20</td>
<td>Singlet</td>
</tr>
<tr>
<td>d</td>
<td>2.158</td>
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<td>Singlet</td>
</tr>
<tr>
<td>e</td>
<td>3.485</td>
<td>2</td>
<td>Singlet</td>
</tr>
</tbody>
</table>

$^a$Spectra was recorded in 90 MHz $^1$H-NMR

The third isolate was resulted at Rf 0.73 as white crystal. Its IR spectra indicated glycoside absorption band at 3400 and 1030 for O-H and C-O stretching vibration. Also provided carbonyl absorption band for carboxyl of glycoside esther group at 1760 (weak). Weak absorption band at 1590 was assigned the existence of conjugated C=C double bond of olefin. And the last, band for methyl, methylene, and methine were absorption at 1940-1840 (2 peaks) and 1390 belong to C-H stretching and bending.

Spectra of $^{13}$C-NMR of triterpenoid aglycone 3 indicated two C=C double bond for this molecule, where recorded at $\delta$ 127.7 and 139.9 positioned at C12-C13 and 134.0 and 137.5 which located at C18-C19. It was provided peak for C3 shifted by hydroxyl group at $\delta$ 90.7 and C28 for carboxyl carbon at $\delta$176.6 ppm. Meanwhile, the other were listed as $\delta$ : 40.2 (C1), 27.5 (C2), 40.3 (C4), 57.2 (C5), 19.3 (C6), 35.8 (C7), 40.3 (C8), 49.0 (C9), 37.8 (C10), 24.2 (C11), 45.7 (C14), 31.6 (C15), 29.7 (C16), 50.8 (C17), 35.6 (C20), 27.2 (C21), 35.8 (C22), 28.6 (C23), 16.7 (C24), 17.1 (C25), 18.7 (C26), 22.3 (C27), 19.8 (C29), 19.3 (C30). The aglycone structure of this molecule was similar to ole-12,18-dienolic acid which was reported by Wu et al. (2007).

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REFERENCES


Masruri, et al.