

# Chemical Constituents from bark of *Euonymus tingen* (Celastraceae)

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## Abstract

Quaillic acid (1) and 6-hydroxy 5-methyl 3',4',5'-trimethoxy aurone 4-O- $\alpha$ -L-rhamnopyranoside (2) were isolated from bark of *Euonymus tingen*. The structures of these compounds were characterized by means of chemical and spectral methods including advanced 2D NMR studies. These compounds were first time isolated from this species.

**Keywords:** *Euonyus tingen*, *Celastraceae*, quaillic acid, aurone

## Introduction

*Euonymus tingen* (Roxb.), vern. Kumkum, belongs to family Celastraceae is a evergreen tree or large shrubs. The bark paste of the plant is useful in eye diseases. Number of Tingenone, hydroxytingenone, triterpenoid quinine methides has been isolated from *Euonymus tingen* [1,2,3]

## Material and Methods:

### *General experimental procedure:*

MPs were uncorrected; UV spectra were taken in MeOH using AlCl<sub>3</sub> as shift reagent. IR recorded in KBr on a Perkin Elmer FT-IR Spectrophotometer. <sup>1</sup>H-NMR were run at 300MHz using TMS as internal standard and C<sub>5</sub>D<sub>5</sub>N and DMSO as solvent. <sup>13</sup>C-NMR recorded in 125MHz, using C<sub>5</sub>D<sub>5</sub>N and DMSO as solvent, FAB-MS on JOEL. JMS700 Mstaion Spectrophotometer.

### **Plant material:**

Stem bark (6 kg) of *Euonymus tingen* was collected from Bheti, Nand Pryag District Chamoli during October 2009 and identified by taxonomist in the Department of Botany, H.N.B. Garhwal University Srinagar. A voucher specimen (GUH-8327) of the plant is deposited at the Herbarium of Department.

### **Extraction and isolation:**

Shade dried and coarsely powdered stem bark of *Euonymus tingen* (4 kg) was extracted thrice with 95% ethanol (5L) at 50°C (15 hrs) on a heating mantle. The reaction mixture was filtered off and the filtrate was concentrated

under reduced pressure to yield brownish residue (440 g). This residue was fractionated with EtOAc (repeatedly 3-4 times) yield EtOAc soluble and insoluble fraction. EtOAc soluble portion (310 g) with n-hexane: Chloroform (93:7) as eluting solvent with increasing polarity of  $\text{CHCl}_3$ , afforded two compounds in pure form tentatively designed as compound **1** and **2** respectively. These compounds were purified by recrystallization.

### Results and discussion:

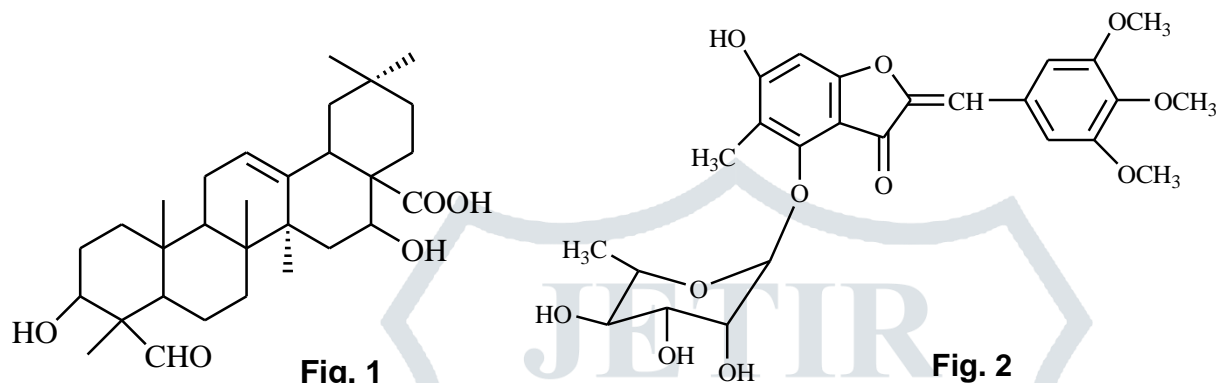
Compound **1** was obtained as an amorphous powder, m.p. 206-207°C, positive to Liebermann-Burchard and Molish reagent. The molecular formula was determined as  $\text{C}_{30}\text{H}_{46}\text{O}_5$  by the LCMS at  $m/z$  486  $[\text{M}]^+$ . Other fragmentation peaks were observed at 442, 414, 398 etc. The mass spectrum of compound also exhibit Retro-Diels Alder fragmentation pattern, their by furnishes the peaks at  $m/z$  246 and 240. The IR spectrum revealed absorption band at  $3419\text{ cm}^{-1}$  (OH),  $1724\text{ cm}^{-1}$  (C=O) and  $1678\text{ cm}^{-1}$  (C=C) of compound showed the presence of thirty carbon signals in the broad band spectrum. In the  $^{13}\text{C}$  NMR spectrum, 6 methyl carbons at  $\delta$  10.2, 15.4, 17.2, 27.1, 33.2 and 24.5 together data with  $^1\text{H}$  NMR spectrum. A broad signal at  $\delta$  5.24 (H-12) in the  $^1\text{H}$  NMR is due to the presence of an olefinic proton which was further supported by  $^{13}\text{C}$  NMR values at  $\delta$  123.60 (C-12) and 145.02 (C-13). Moreover, two downfield signals at  $\delta$  174.8 (C-28) and 206.4 (C-23) were clearly indicative to carbonyl carbons [4]. Compound **1** has been identified as **Quaillic acid** (Fig.1) on the basis of spectral data [IR,  $^{13}\text{C}$ ,  $^1\text{H}$ -NMR and Mass] and reported literature [5].

Compound **2** was crystallized as yellow crystals from  $\text{CHCl}_3:\text{MeOH}$ , m.p: 192-193°C. The LCMS of compound showed molecular ion peak at  $m/z$  520  $[\text{M}]^+$ , which corresponds the molecular formula  $\text{C}_{25}\text{H}_{28}\text{O}_{12}$ . The other fragmentation peaks were observed at  $m/z$  358, 357, 341, 330, 192, 138 and 92. The aglycone  $\text{C}_{19}\text{H}_{18}\text{O}_7$  was found to be aurone on the basis of colour reaction and UV absorption band  $\lambda_{\text{max}}$  (nm) at 260, 330, 405 [6]. Aglycone was analysed for three methoxyl groups and their presence was confirmed by a singlet at  $\delta$  3.63 (9H). NMR showed presence of methoxyl groups at 3', 4' and 5' position.  $^1\text{H}$ -NMR studies of the aglycone showed the presence of three aromatic protons suggesting a hexa-substituted aurone. A multiplet at  $\delta$  7.88-8.00 (2H) was due to C-2' and C-6' protons and a singlet at  $\delta$  6.23 (1H) for C-7 protons. A singlet at  $\delta$  1.52 (3H) and  $\delta$  6.75 (1H) was assignable to 3H of Me group and benzyne proton (-CH-) respectively. The above position of various groups was further confirmed by  $^{13}\text{C}$  NMR spectral analysis.

On acetylation aglycone formed diacetate, m.p. 104°C indicating the presence of two hydroxyl groups. Mass spectral data showed a molecular ion peak at 520. Two fragments of  $m/z$  192 and 166 showed that three methoxyl groups were present in the ring B, two hydroxyl and one methyl group in the ring A. The positions of hydroxyl were shown to be at C-4 and C-6 of glycone by UV spectral shifts were C-5 and C-7 for attachment of methyl group in the ring A. C-5 position for methyl group was confirmed by  $^1\text{H}$  NMR and  $^{13}\text{C}$ -NMR .

Attachment of the sugar at C-4 of the aglycone was determined by comparing the  $^{13}\text{C}$  NMR spectra of the glycoside with that of aglycone. No change in  $\lambda_{\text{max}}$  with the addition of  $\text{AlCl}_3$ , confirmed that it was a 4-O-glycoside. The sugar was identified as rhamnose by paper co-chromatography with authentic sample and by  $^1\text{H}$ -

NMR spectral study of glycoside, a doublet at  $\delta$  1.10 corresponding to 3H of rhamnosyl -CH<sub>3</sub>, broad signal at  $\delta$  3.5-3.8 for 4H and a singlet at  $\delta$  4.2 due to -C-1'' protons of rhamnose. This led to the formulation of the compound B-5 as 6-hydroxy-5,3',4',5'-tetramethoxy aurone 4-O- $\alpha$ -L-rhamnopyranoside. The sugar Structure of compound B-5 was also confirmed by comparative study with the authentic sample (Kesari et al., 2004). The compound has been identified as **6-hydroxy 5-methyl 3',4',5'-trimethoxy aurone 4-O- $\alpha$ -L-rhamnopyranoside** (Fig.2).



**Table.** <sup>13</sup>C (125 MHz) and <sup>1</sup>H NMR (300 MHz) data of compound 1 and 2 in Pyridine (C<sub>5</sub>D<sub>5</sub>N) and DMSO-d<sub>6</sub>

Position(1)	$\delta_c$	$\delta_H$ J(Hz)	Position(2)	$\delta_c$	$\delta_H$ J(Hz)
1	37.9	1.03 (t,13.3)	2	154.4	-
		1.81(d, 13.3,3.4)	3	190.4	-
2	25.1	1.72, 1.47m	4	162.1	-
3	81.9	4.11 (t,8.5)	5	120.4	-
4	55.4	-	6	161.8	-
5	46.9	1.50, 1.60 m	7	94.6	6.0s
6	20.3	1.60, 1.42 m	8	160.8	-
7	32.6	-	9	105.4	-
8	39.9	1.72 (dd, 11.7, 6.5)	10	115.2	6.3s
9	47.1	0.74 (d, 11.4)	1'	122.1	6.7s
10	37.9	-	2'	115.6	7.7s
11	23.5	2.10m	3'	158.7	-
12	120.9	5.82s	4'	154.2	-
13	143.0	-	5'	158.7	-
14	42.0	-	6'	115.6	8.5s
15	35.9	1.52, 1.27m	CH <sub>3</sub>	23.8	1.9s
16	73.10	5.24 br	O-CH <sub>3</sub>	58.6	4.0s
17	48.6	-	Rha		
18	41.2	3.63 (dd, 14.5, 4.8)	1''	101.4	4.2s
19	47.5	2.85 (t, 14.0)	2''	72.6	3.25
20	30.9	-	3''	72.3	3.4
21	35.8	1.49 (dd, 13.3 3.2)	4''	73.7	3.6
		1.24 (dd, 13.3, 3.2)	5''	70.4	3.8
22	32.8	1.75, 1.50m	6''	18.8	1.1(d,13.3)
23	206.4	9.03s			
24	10.2	1.07s			
25	15.4	0.91s			
26	17.2	1.03s			

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27	27.1	1.19s
28	174.8	11.02s
29	33.2	1.25s
30	24.5	1.21s
OH-3	-	5.92s
OH-16	-	6.23s

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