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## Phytochemical Investigations on *Elaeagnus Umbellata*

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## PHYTOCHEMICAL INVESTIGATIONS ON ELAEAGNUS UMBELLATA

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# Phytochemical Investigations on *Elaeagnus umbellata*

Syeda Farina Asghar<sup>a</sup> & Habib-ur-Rehman<sup>o</sup>

*Abstract* - In the course of phytochemical investigations on methanol extract of *Elaeagnus umbellata* lead to the isolation of new isoflavone and phenol compound as a source from this plant. The isolation of plant also gave the terpenoid compound. The name of isolated compounds are; 3-(hydroxymethyl)-4-methoxyphenol (1) and 5, 7-dihydroxy-3(2-hydroxyphenyl-4//-chromen-4-one (1) and Stigmasterol (3). This is the first report of the isolation of compounds (1) and (2). The Characterization of the compounds was made on the basis of spectral studies.

*Keywords* : phytochemical, isoflavone, phenol and spectral studies.

#### I. INTRODUCTION

Laeagnus umbellata is a key plant for the font of indole 3-carbinol alkaloid (Tolkachev & etal.<sup>1</sup>). 'Cervical cancer' is reduced by indole 3-carbinol alkaloid (it is an anti-estrogenic) (Yuan & etal.<sup>2</sup>). For the plant growth and development chitinases play vital role and *Elaeagnus umbellata* is rich in endochitinases in their root nodules (Yaw Joo & etal.<sup>3</sup>).The plant have effective antibacterial activity (Sabir & etal <sup>4</sup>).Vitamins A, C and E, flavonoids, isoflavonoids, essential fatty acids, acids, lycopene, β-carotene, lutein, phytofuene are abundant in the berries of the plant (Chopra, Kohlmeier, Fordham & etals<sup>5-7</sup>).

#### II. EXPERIMENTAL

*Instrumentation :* The instruments used during research for collecting data are listed in table A.

#### a) Plant Collection

The collection of plant material was done at Athmaqam District Neelum Valley Muzaffarabad Azad Kashmir Pakistan. The identification of plant was done by the help of taxonomist at the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad. The voucher specimen has been kept in the herbarium of the department. The isolation of compounds is given in the scheme A.

i. Compounds isolated from Elaeagnus umbellata

Isolation and Characterization of Compound 1:

The flash column chromatography was used for

the purification of fraction U4. The column was eluted with Ethyl acetate/ Chloroform (2.0:8.0) as the solvent system to afford two fractions, U4-1 and U4-2. The fraction U4-1 was rechromatographed on the precoated silica-gel (GF-254) plates with Chloroform/Ethyl acetate (2.0:8.0) as the solvent system. That resulted in the isolation of the pure compound **1** as an amorphous material (20mg, Rf = 0.6).

#### Spectral Data:

#### UV (MeOH) λmax (nm): 202, 223, and 276.

IR (CHCl<sub>3</sub>) υ<sub>max</sub>, (cm<sup>-1</sup>): 3405 (O-H), 2925 (aromatic C-H) and 1031 (C-O).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300MHz)  $\delta$ : 3H (s)  $\delta$  3.83 (4-OCH<sub>3</sub>) 2H (s)  $\delta$  4.61 (3-CH<sub>2</sub>OH), 1H (s)  $\delta$  6.99 (2-H),1H (d)  $\delta$  6.75 (*J*= 8.2 Hz) (5-H), 1H (d)  $\delta$  6.93 (*J*= 8.2 Hz) (6-H), 1H (t)  $\delta$  5.35 (*J*= 4.0 Hz) (1-OH), 1H (t)  $\delta$  3.65 (*J*= 4.0 Hz) (3-OH).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100MHz) δ: 1-C (δ 154.7), 2-C (δ 116.8), 3-C (δ 135.7), 4-C (δ 149.3), 5-C (δ 112.7), 6-C (δ 115.8), 3-CH<sub>2</sub>OH (δ 60.5), 4-OCH<sub>3</sub>(δ 56.1).

**HRMS m/z**: 154.020 ( $C_8H_{10}O_3$ ), 153, 137 and 123.

#### Isolation and Characterization of Compound 2:

The fraction U4-2 was rechromatographed on the precoated silica-gel (GF-254) plates with Chloroform/Ethyl acetate (3.0:7.0) as the solvent system. That resulted in the isolation of the pure compound **2** as amorphous material (20mg, Rf = 0.9).

#### Spectral Data:

**UV (MeOH)** *λ*<sub>max</sub> (nm): 203, 215 and 264.

IR (CHCl<sub>3</sub>)  $\upsilon_{max}$  (cm<sup>-1</sup>): 3430 (O-H), 2953 (aromatic C-H), 1659 ( $\alpha$ ,  $\beta$ -unsaturated C=O) and 1064 (C=O).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400MHz)  $\delta$ : 1H (s)  $\delta$  7.8 (2-H), 1H (s)  $\delta$  6.0 (6-H),1H (s)  $\delta$  6.2 (8-H), 1H (d)  $\delta$  6.7 (J = 8.4 Hz) (3'-H), 1H (d)  $\delta$  7.0 (J = 8.2 Hz) (6'-H), 1H (t)  $\delta$ 7.1 (J = 10.0 Hz) (4'-H), 1H (t)  $\delta$  6.9 (J = 10.0 Hz) (5'-H), 3H (br)  $\delta$  12.5 (5-OH), (7-OH) and (2'-OH) respectively.

<sup>13</sup>C-NMR (CDCl3, 100MHz) δ: 2-C ( $\delta$  153.2), 3-C ( $\delta$  121.7), 4-C ( $\delta$  180.7), 5-C ( $\delta$  161.8), 6-C ( $\delta$  98.3), 7-C ( $\delta$  166.4), 8-C ( $\delta$  94.0), 9-C ( $\delta$  160.0), 10-C ( $\delta$  105.5), 1-C (120.2), 2-C ( $\delta$  156.6), 3-C ( $\delta$  117.6), 4-C(129.3), 5-C

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(121.2) and 6<sup>-</sup>C (130.1).

HRMS, m/z: 270.060 ( $C_{15}H_{10}O_5),\ 269,\ 253,\ 176$  and 76.

#### Isolation and Characterization of Compound 3:

The flash column chromatography was used for the purification of fraction U5. The column was eluted with Ethyl acetate that afforded two fractions, U5-1 and U5-2. The fraction U5-2 was rechromatographed on the precoated silica-gel (GF-254) plates with Chloroform/ Ethyl acetate (1.0:9.0) as the solvent system. That resulted in the isolation of the pure compound **3** as amorphous material (20mg, Rf = 0.7).

#### Spectral Data:

UV (MeOH) λ<sub>max</sub> (nm): 203, 215 and 264.

IR (CHCl<sub>3</sub>) v<sub>max</sub> (cm<sup>-1</sup>): 3484 (O-H), 2868 (C-H), 1659 and (C=C).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400MHz) δ: 3H (s) δ 0.69 (18-CH<sub>3</sub>), 3H (s) δ 0.99 (19-CH<sub>3</sub>), 3H (t) δ 0.78(J=7.5Hz) (29-CH<sub>3</sub>),3H (d) δ 1.00 (J= 6.5 Hz) (21-CH<sub>3</sub>), 3H (d) δ 0.82 (J= 6.0 Hz) (26-CH<sub>3</sub>), 3H (d) δ 0.77 (J= 6.0 Hz) (27-CH<sub>3</sub>), 1H (bs) δ 5.23 (6-H).

<sup>13</sup>C-NMR (CDCl3, 100MHz)  $\delta$ : 1-C ( $\delta$ 37.2), 2-C ( $\delta$ 31.6), 3-C ( $\delta$  $\delta$ 71.8), 4-C ( $\delta$ 42.2), 5-C ( $\delta$ 140.8), 6-C ( $\delta$ 121.7), 7-C ( $\delta$ 31.9), 8-C ( $\delta$ 31.9), 9-C ( $\delta$ 51.2), 10-C (36.5), 11-C (21.1), 12-C ( $\delta$ 39.8), 13-C ( $\delta$ 42.3), 14-C(56.9), 15-C (24.3) 16-C (28.4) 17-C ( $\delta$ 56.1), 18-C ( $\delta$ 11.2), 19-C ( $\delta$ 21.4), 20-C ( $\delta$ 40.2),21-C ( $\delta$ 21.3), 22-C ( $\delta$ 138.4), 23-C ( $\delta$ 129.2) 24-C ( $\delta$ 51.2), 25-C ( $\delta$ 31.7), 26-C ( $\delta$ 21.2), 27-C ( $\delta$ 19.0), 28-C ( $\delta$ 25.4) and 29-C ( $\delta$ 12.2).

HRMS, m/z: 412.3861 (C $_{\rm 29}H_{\rm 48}O),~413,~396,~283$  and 60.

#### III. Results and Discussions

#### a) 3-(hydroxymethyl)-4-methoxyphenol (1)

The UV spectrum (MeOH) of compound (1) showed the  $\lambda_{max}$  absorptions at, 203 nm 223 nm and 276 nm, suggesting the phenol type compound. The IR spectrum (CHCl<sub>3</sub>) of compound (1) showed intense  $v_{max}$  absorptions at 3405 cm<sup>-1</sup>, 2925 cm<sup>-1</sup>, and 1031 cm<sup>-1</sup>, indicating the presence of O-H, aromatic C-H and C-O functions in the molecule.

The <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400MHz) spectrum of compound (1) showed the presence of 10 proton resonances in the molecule. The spectrum showed 3H singlet at  $\delta$  3.83, assigned to the methoxy protons (4-OCH<sub>3</sub>) of the compound. Another singlet of 2H centred at  $\delta$  4.61 given to the methylene protons (3-CH<sub>2</sub>OH). 1H singlet appeared at  $\delta$  6.99 given to 2-H of benzene proton. Two doublets of 1H each appeared at  $\delta$  6.75 (*J*= 8.2 Hz) and  $\delta$  6.93 (*J*= 8.2 Hz) were assigned to the 5-H and 6-H benzene protons respectively. Two triplets of 1H each appeared at  $\delta$  3.65

The <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz) spectrum of the compound **(1)** showed the presence of 8 carbon atoms in the molecule. The <sup>13</sup>C-NMR chemical shift assignments made by DEPT pulse sequences are presented in Table-2. The downfield signal at  $\delta$  154.7 showed the multiplicity of one carbon assigned to the 1-C attached to the hydroxyl group. The signals at  $\delta$  116.8,  $\delta$  135.7,  $\delta$  149.3,  $\delta$  112.7 and  $\delta$  115.8 were assigned to the 2-C, 3-C, 4-C, 5-C and 6-C carbon atoms of benzene ring respectively. The signal at  $\delta$  56.1 was given to the 4-OCH<sub>3</sub>. The signal appeared at  $\delta$  60.5 assigned to the methylene carbon attached to hydroxyl group 3-CH<sub>2</sub>OH.

The mass spectrum of the compound (1) showed the molecular ion peak at m/z 154.020, corresponding to the molecular formula  $C_8H_{10}O_3$  suggesting the presence of four degrees of unsaturation in the molecule. The prominent peaks were found to occur at m/z 154, 153, 137and 123. The peak appeared at m/z 153 showed the loss of hydrogen atom from the molecule. The peak at m/z 137 showed the loss of hydroxyl group from the molecular ion. The peak at m/z 123 showed the loss of spectral data it is confirmed that the compound (1) is two substituted phenol namely; 3-(hydroxymethyl)-4-methoxyphenol.



#### b) 5, 7-dihydroxy-3(2-hydroxyphenyl-4H-chromen-4one(2)

The UV spectrum (MeOH) of the compound **3** showed the  $\lambda_{\text{max}}$  absorptions at 203 nm, 215 nm and 264 nm, representing the isoflavone type structure of the molecule. The IR spectrum (CHCl<sub>3</sub>) showed intense absorptions at 3430 cm<sup>-1</sup>, 2953 cm<sup>-1</sup>, 1659 cm<sup>-1</sup> and 1064 cm<sup>-1</sup>, indicating the presence of O-H, aromatic C-H,  $\alpha$ ,  $\beta$ -unsaturated C=O and C-O functionalities in the molecule.

The <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300MHz) spectrum of the compound **2** showed 10 proton resonances in the molecule. Three singlets of 1H each appeared at  $\overline{\delta}$  7.8,  $\overline{\delta}$  6.0 and  $\overline{\delta}$  6.2 were assigned to the 2-H, 6-H and 8-H respectively. The protons of 3'-H and 6'-H appeared as 1H doublets each at  $\overline{\delta}$  6.7 (*J*= 8.4) and  $\overline{\delta}$  7.0 (*J*= 8.2 Hz), respectively. Two triplets of 1H each viewed at  $\overline{\delta}$  7.1 (*J*= 10.0) and  $\overline{\delta}$  6.9 (*J*= 10.0) given to the 4'-H and 5'-H protons respectively. The broad singlet of 3H at  $\overline{\delta}$  12.5 showed the presence of hydroxyl protons in the compound and the peak assigned to the 5-OH, 7-OH and 2'-OH protons respectively. The 'H-NMR chemical shifts of compound **2** are presented in Table-3.

The <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz) spectrum of the compound **(3)** showed the presence of 15 carbon atoms in the molecule. The <sup>13</sup>C-NMR (DEPT) chemical shift assignments presented in Table-4. The most

downfield signal at  $\overline{\delta}$  180.7 showed the multiplicity of one carbon and assigned to the 4-C carbonyl carbon. The other carbon signals at  $\overline{\delta}$  153.2,  $\overline{\delta}$  121.7, 161.8,  $\overline{\delta}$  98.3,  $\overline{\delta}$  166.4  $\overline{\delta}$  94.0,  $\overline{\delta}$  160.0,  $\overline{\delta}$ 105.5,  $\overline{\delta}$  120.2,  $\overline{\delta}$  156.6,  $\overline{\delta}$  117.6,  $\overline{\delta}$  129.3,  $\overline{\delta}$  121.2 and  $\overline{\delta}$  130.1 were given to the 2-C, 3-C, 5-C, 6-C, 7-C, 8-C, 9-C, 10-C, 1-C, 2-C, 3-C, 4-C, 5-Cand 6-C.

The mass spectrum of the compound **2** showed the molecular ion peak at m/z 270.060, corresponding to the molecular formula  $C_{15}H_{10}O_5$ , showed the 11 degrees of unsaturation in the molecule, Other prominent peaks were found to occur at m/z 269, 253, 176 and 77. The peak at m/z 269 indicated the loss of hydrogen atom while the peak at m/z 253 showed the loss of hydroxyl group from the molecular ion. The peak at m/z 176 suggested the loss of phenol from the molecule



#### c) Stigmasterol (3)

The UV spectrum (MeOH) showed the  $\lambda_{max}$  absorptions at 195nm, 204 nm and 387 nm, representing a steroid skeleton. The IR spectrum (CHCl<sub>3</sub>) showed intense  $v_{max}$  absorptions at 3374 cm<sup>-1</sup>, 2936, 1462 cm<sup>-1</sup> and 1058 cm<sup>-1</sup>, indicating the presence of O-H, C-H, C=C and C-O functions in the molecule.

The <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400MHz) spectrum of **3** showed the presence of 48 proton resonances in the molecule. The spectrum showed two 3H singlets each at  $\delta$  0.69 and  $\delta$  0.99 given to the 18-CH<sub>3</sub> and 19-CH<sub>3</sub>, respectively. A 3H triplet at  $\delta$  0.78 (*J*=7.5Hz) was assigned to the 29-CH<sub>3</sub>. Three doublets of 3H each at  $\delta$  1.00 (*J*= 6.5 Hz),  $\delta$  0.82 (*J*=6.0 Hz) and  $\delta$  0.77 (*J*=6.0 Hz) assigned to the 21-CH<sub>3</sub>, 26-CH<sub>3</sub> and 27-CH<sub>3</sub>, respectively. A broad singlet of 1H appeared at  $\delta$  5.23 was assigned to the 6-H olefinic proton. The <sup>1</sup>H-NMR chemical shift assignments are presented in Table-5.

The <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 400 MHz) spectrum of the compound **3** showed the presence of 29 carbon atoms in the molecule. The <sup>13</sup>C-NMR chemical shift assignments determined by DEPT pulse sequence are

presented in Table-6. The signals at  $\delta$  37.2  $\delta$  31.6,  $\delta$  71.8,  $\delta$  42.2,  $\delta$  140.8,  $\delta$  121.7 and were assigned to the 1-C, 2-C, 4-C, 3-C, 5-C and 6-C respectively. The signal of two carbons multiplicity at  $\delta$  31.9 given to the 7-C and 8-C. The signals at  $\delta$  51.2,  $\delta$  36.5,  $\delta$  21.1,  $\delta$  39.8,  $\delta$  42.3, 56.9,  $\delta$  24.3,  $\delta$  28.4,  $\delta$  56.1,  $\delta$  11.2,  $\delta$  21.4,  $\delta$  40.2 and  $\delta$  21.3 and were assigned to the 9-C. and 10-C, 11-C, 12-C, 13-C, 14-C, 15-C, 16-C, 17-C, 18-C, 19-C, 20-C and 21-C respectively. The signals at  $\delta$  138.4 and  $\delta$  129.2 assigned to the 22-C and 23-C unsaturated carbons respectively. The signals at  $\delta$  51.2,  $\delta$  31.7,  $\delta$  21.2,  $\delta$  19.0,  $\delta$  25.4 and  $\delta$  12.2 were assigned to the 24-C, 25-C, 26-C, 27-C, 28-C and 29-C respectively.

The mass spectrum of the compound **3** showed the molecular ion peak at m/z 412.3861, corresponding to the molecular formula  $C_{29}H_{48}O$ , indicating five degrees of unsaturation in the molecule. Other prominent peaks were found to occur at m/z, 413, 396, 283 and 60. The peak at m/z 413 indicated the loss of hydrogen atom while the peak at m/z 396 showed the loss of water molecule from the molecular ion (Pateh *et a*/<sup>8</sup>)



(3)

#### Table A : list of instruments used during research.

S.#	Data	Instruments
1.	UV spectra	Shimadzu UV-240
2.	IR spectra	JASCO A-302 spectrophotometer
3.	High resolution mass spectra	MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system
4.	<sup>1</sup> H- NMR spectra	400 MHz on a Bruker AM-300 NMR spectrometer
5.	<sup>13</sup> C-NMR spectra	100 MHz on a Bruker AM-300 NMR spectrometer
6.	TLC	silica gel plates (GF-254, 0.2 mm, E.Merck)



Scheme A : Isolation of compounds.

<i>Table-1 :</i> <sup>1</sup> H-NMR	(CDCl <sub>3</sub> , 400MHz)	Chemical	Shift Assignments	of Compound 1.	

Proton No.	Chemical Shift (δ) (ppm)	Integration	Multiplicity	Coupling Constant (J) (Hz)
4-OCH <sub>3</sub>	3.83	ЗH	S	-
3-CH <sub>2</sub> OH	4.61	2H	S	-
2-H	6.99	1H	S	-
5-H	6.75	1H	d	8.2
6-H	6.93	1H	d	8.2
1-OH	5.35	1H	t	4.0
3-OH	3.65	1H	t	4.0

*Table-2*:<sup>13</sup>C-NMR (CDCI<sub>3</sub>, 400 MHz) Chemical Shift Assignments of Compound 1.

Carbon No.	Chemical Shift (δ) (ppm)	Carbon No.	Chemical Shift (δ) (ppm)
1-C	154.7	5-C	112.7
2-C	116.8	6-C	115.8
3-C	135.7	3-CH <sub>2</sub> OH	60.5
4-C	149.3	4-OCH <sub>3</sub>	56.1

#### *Table-3*:<sup>1</sup> H-NMR (CDCl<sub>3</sub>, 400MHz) Chemical Shift Assignments of Compound 2.

Proton No.	Chemical Shift (δ) (ppm)	Integration	Multiplicity	Coupling Constant (J) (Hz)
2-H	7.8	1H	S	-
6-H	6.0	1H	S	-
8-H	6.2	1H	S	-
3'-H	6.7	1H	d	8.4
6'-H	7.0	1H	d	8.2
4'-H	7.1	1H	t	10.0
5'-H	6.9	1H	t	10.0
5,7,2 <sup>-</sup> OH	12.5	ЗH	bs	-

Table-4 :<sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz) Chemical Shift Assignments of Compound 2.

Carbon No.	Chemical Shift ( $\delta$ ) (ppm)	Carbon No.	Chemical Shift (δ) (ppm)
2-C	153.2	10-C	105.5
3-C	121.7	1'-C	120.2
4-C	180.7	2-C	156.6
5-C	161.8	3'-C	117.6
6-C	98.3	4'-C	129.3
7-C	166.4	5'-C	121.2
8-C	94.0	6-C	130.1
9-C	160.0	-	-

Table-5:<sup>1</sup> H-NMR (CDCl<sub>3</sub>, 400MHz) Chemical Shift Assignments of Compound 3.

Proton No.	Chemical Shift (δ) (ppm)	Integration	Multiplicity	Coupling Constant (J) (Hz)
18-CH <sub>3</sub>	0.69	ЗH	S	-
19-CH <sub>3</sub>	0.99	ЗH	S	-
29-CH <sub>3</sub>	0.78	ЗH	t	7.5
21-CH₃	1.00	ЗH	d	6.5
26-CH <sub>3</sub>	0.82	ЗH	d	6.0
27-CH <sub>3</sub>	0.77	ЗH	d	6.0
6-H	5.23	1H	bs	-

Carbon No.	Chemical Shift ( $\delta$ ) (ppm)	Carbon No.	Chemical Shift $(\delta)$
1-C	37.2	16-C	28.4
2-C	31.6	17-C	56.1
3-C	71.8	18-C	11.2
4-C	42.2	19-C	21.4
5-C	140.8	20-C	40.2
6-C	121.7	21-C	21.3
7-C	31.9	22-C	138.4
8-C	31.9	23-C	129.2
9-C	51.2	24-C	51.2
10-C	36.5	25-C	31.7
11-C	21.1	26-C	21.2
12-C	39.8	27-C	19.0
13-C	42.3	28-C	25.4
14-C	56.9	29-C	12.2
15-C	24.3		

*Table-6* :<sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz) Chemical Shift Assignments of Compound **3**.

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