Isolation of Flavones from Indigenous *Dodonaea viscosa*

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Summary: Here in we report the isolation and structure elucidation of pure compounds from chloroform and ethyle acetae soluble fractions of *Dodonaea viscose*. The chloroform soluble fraction yielded 6*β*-hydroxy-15, 16-epoxy-5*β*, 8*β*, 9*β*, 10*α*-celoda-3, 13(16), 14-trien-18-oic acid **1**, 2*β*-hydroxy-15, 16-epoxy-5*β*, 8*β*, 9*β*, 10*α*-celoda-3, 13(16), 14-trien-18-oic acid **2**, 3,5,7-trihydroxy 6,4′-dimethoxyflavanone **3** while ethyl acetate fraction resulted 3,5,4′-trihydroxy-6,7-dimethoxyflavone **4** and 5,4-dihydroxy-3,6,7-trimethoxyflavone **5**. Structures of all the isolated compounds were established with the help of spectroscopic techniques including; UV, IR, Mass, 1D and 2D NMR spectroscopy.

Key words: *Dodonaea viscose;* chloroform soluble fraction; ethyl acetate soluble fraction; flavonoids.

Introduction

Dodonaea viscosa is a well-known indigenous medicinal plant used in folk medicine to treat various ailments; leaves of this plant have been found effective against sore throat, wounds, fever (piles); fever, malaria, angina, cold, arthritis, sinusitis flu. and boils, dressing for skin diseases of the head and face. Previous investigation shows that the compounds isolated from this plant exhibited antibacterial, antiviral, antifungal and anti-inflammatory activities [1-6]. Phytochemical investigation of *Dodonea viscose* shows that this plant is rich in flavonoids, saponins and diterpenes [7-16]. Therapeutical importance of the *Dodonea viscosa* thus prompted us to carry out phytochemical investigations on indigenous specie collected from Kurram Agency, Khyber Pukhtunkhwa, Pakistan.

Results and Discussion

Compound **1** (Fig. 1) was isolated as colorless gummy solid. The HR-EIMS of compound **1** provided $[M]^+$ peak at *m/z* 332.1990 (calc. for $C_{20}H_{28}O_4$, 332.1987) establishing the molecular formula as $C_{20}H_{28}O_4$. The UV spectrum showed an absorption band at λ_{max} 212 nm. The IR spectrum revealed the presence of hydroxyl group (3336 cm^{-1}), carbonyl group (1680 cm^{-1}) and a furan ring (1505 and 876 cm^{-1}). The mass spectrum showed peaks at *m/z* 95, 94, 81 suggesting the presence of furan ring with an alkyl chain. Similarly other fragments at *m/z* 219, 201, 173 indicated the presence of diterpenoid skeleton with a hydroxyl group (Scheme 1).

The ^{1}H-NMR spectrum of **1** closely resembled to those of *trans*-clerodanes revealed the same substitution pattern in rings A and B. It include signals for two tertiary methyl groups at δ 0.70 and 1.17, one secondary methyl at δ 0.82 (3H, d, J = 6.6 Hz) and an olefinic proton at C-3 position at δ 7.01 (1H, dd, J = 3, 4.7 Hz). A double doublet at δ 3.63 (1H, J = 4.8, 11.0 Hz) was assigned to the geminal proton of a secondary hydroxyl group at H-6. Typical downfield signals at δ 7.30 (1H, t, J = 1.67 Hz), 7.15 (1H, dd, J = 0.9, 1.4 Hz) and 6.20 (1H, dd, J = 0.8, 1.7 Hz) were assigned to H-15, H-16 and H-14, respectively, suggesting the presence of 3-substituted furan ring.

The ^{13}C-NMR spectrum (BB and DEPT) confirmed the presence of three methyl, five methylene, seven methine and five quaternary carbon atoms. The ^{13}C-NMR chemical shift of Me-19 was observed at δ 16.5. The *β*–positioned and axial Me-20 appeared at δ 17.2, while *β*–positioned and equatorial Me-17 resonated at δ 15.2. These values supported the presence of *trans* configuration at the A/B ring junction of **1 (**Table-1).

The position of hydroxyl group at C-6 was confirmed by HMBC experiments which showed ^2J correlation of Me-19 (δ 1.17) to C-5 (δ 45.4) and ^3J correlations to C-6 (δ 74.2), C-4 (δ 140.7), and C-10 (δ 45.4). The position of carboxylic acid at C-4 was also confirmed by HMBC experiments which showed ^2J correlations of H-3 (δ 7.0) to C-4 (δ 140.7) and ^3J correlations to C-5 (δ 45.5) and C-18 (δ 172.2). The AB pattern centered at δ 1.56 and δ 1.64 could be assigned as CH_2-11; the HMBC showed ^2J correlations to C-9 (δ 38.4) and C-12 (δ 17.2) and ^3J to C-8 (δ 33.6), C-10 (δ 45.4), and C-20 (δ 17.2), confirming the attachments of alkyl chain to C-9 (Fig. 2).

A series of nuclear *Overhauser* Effect (NOE) experiments were carried out for **1** to

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differentiate between Me-19 and Me-20 as well as Me-17 and Me-20, consistent with a *cis* relationship between these methyl groups. These results and the fact the irradiation of H-10 did not cause any increase in the intensities of either Me-19-or Me-20 signals confirmed the *trans* configuration of the A and B rings of the decalin systems of **1** (Fig. 3). No NOE was observed between the H-16 and both CH_3-19 and CH_3-20 indicated their *trans* relationship and establishing the *β*–configuration and equatorial confirmation of hydroxyl at C-6. This was also confirmed by the coupling constants of H-6 and the inspection of Dreiding model. The compound **1** was therefore, assigned the structure as 6*β*-hydroxy-15,16-epoxy-5*β*, 8*β*, 9*β*, 10*α*-celoda-3, 13(16), 14-trien-18-oic acid.

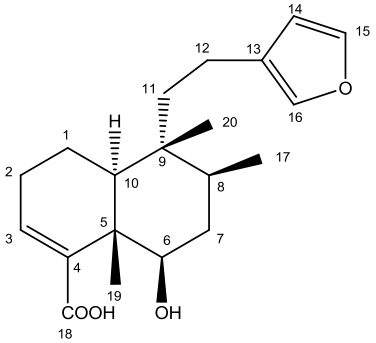
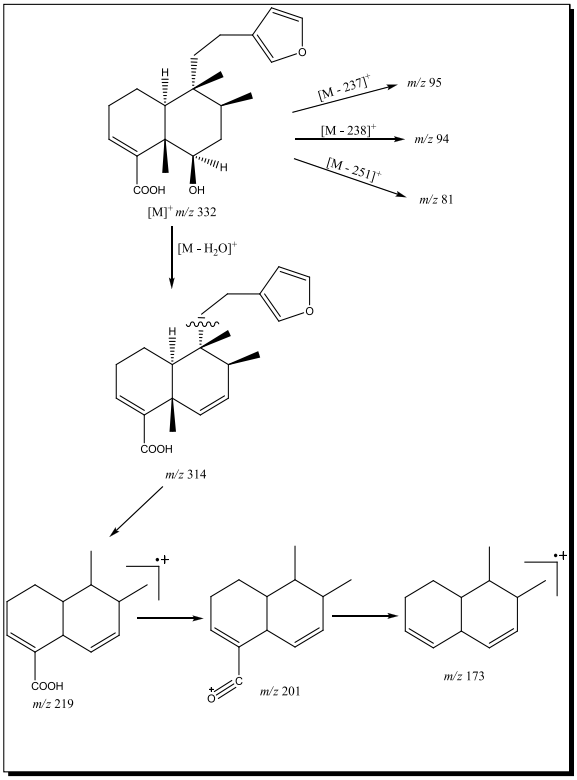


Fig. 1: Structure of compound **1**.



Scheme-1: Mass Fragmentation pattern of **1**.

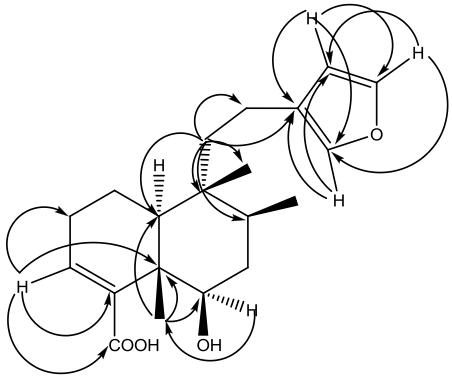


Fig. 2: Important HMBC correlations of compound **1**.

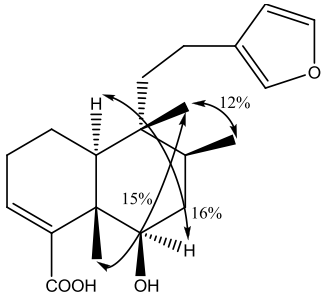


Fig. 3: Important NOE interactions of compound **1**.

Table-1: ^{13}C-NMR spectral data of **1**.

Carbon No.	(*δ*) C-MR (1)	Multiplicity	Carbon No.	(*δ*) C-MR (1)	Multiplicity
1	17.0	CH_2	11	38.4	CH_2
2	27.8	CH_2	12	17.2	CH_2
3	142.0	CH	13	125.0	-C-
4	140.7	-C-	14	110.6	CH
5	45.5	-C-	15	142.4	CH
6	74.2	CH	16	138.0	CH
7	35.5	CH_2	Me-17	15.2	CH_3
8	33.6	CH_2	18	172.2	-C-
9	38.4	-C-	Me-19	16.5	CH_3
10	45.5	CH	Me-20	17.2	CH_3

Table-4: One bond HMQC interaction of **1**.

Positions	^{1}H-NMR (*δ*)	^{13}C-NMR (*δ*)
1	1.51 (m), 1.68 (m)	17.0
2	2.19 (m), 2.30 (m)	27.8
3	7.01 (dd, *J* = 3, 4.7 Hz)	142.0
4	--	140.7
5	--	45.5
6	3.63 (dd, *J* = 4.8, 11.0 Hz)	74.2
7	1.62 (m)	35.5
8	--	33.6
9	--	38.4
10	1.32 (br d, *J* = 12.0 Hz)	45.5
11	1.56 (ddd, *J* = 14.2, 12.1, 5.0 Hz) 1.64 (ddd, *J* = 14.2, 12.1, 5.2 Hz)	38.4
12	2.15 (ddd, *J* = 14.2, 12.1, 5.2 Hz) 2.25 (ddd, *J* = 14.2, 12.1, 5.0 Hz)	17.2
13	--	125.0
14	6.20 (dd, *J* = 0.8, 1.7 Hz)	110.6
15	7.30 (t, *J* = 1.67 Hz)	142.4
16	7.15 (dd, *J* = 0.9, 1.4 Hz)	138.0
Me-17	0.82 (d, *J* = 6.6 Hz)	15.2
Me-18	--	172.2
Me-19	1.17 (s)	16.5
Me-20	0.70 (s)	17.2

Compound **2** (Fig. 4) was isolated as colorless gummy solid having $[\alpha]_D = 100^o$. The HR-EIMS of **2** provided $[M]^+$ peak at *m/z* 332.1983 (calc. for $C_{20}H_{28}O_4$, 332.1987) indicating the molecular formula as $C_{20}H_{28}O_4$. The peaks at *m/z* 94, 95, 81 resulted from the cleavage of the furanyl alkyl moiety suggesting the presence of furan ring with alkyl chain (**Scheme 2)**. The UV spectrum showed absorption band at λ_{max} 204 nm while the IR spectrum revealed the presence of a hydroxyl group (3336 cm^{-1}), carbonyl group (1680 cm^{-1}) and furan ring (1505 and 876 cm^{-1}). A comparison of ^{1}H-NMR and ^{13}C-NMR spectra of **2** with **1** revealed a close similarity between the two compounds.

The ^{1}H-NMR spectrum of compound **2** showed a signal at δ 4.30 (m, 1H) indicates that one of the two protons at H-2 has been substituted by a hydroxyl group. While signals appeared for two tertiary methyl groups at δ 1.22 and δ 0.77 and one secondary methyl at δ 0.82 (3H, d, J = 6.5 Hz). The broad singlet at δ 6.11 was ascribed as olefinic H-3 proton. Typical downfield signals in the ^{1}H-NMR spectrum of **1** at δ 7.32 (1H, t, J = 1.7 Hz), 7.20 (1H, br. s) and 6.26 (1H, dd, J = 0.8, 1.7 Hz) were attributed to H-15, H-16 and H-14, respectively, suggesting the presence of 3-substituted furan ring.

The ^{13}C-NMR spectrum (BB and DEPT) of compound **2** indicated the presence of three methyl, five methylene, seven methine and five quaternary carbon atoms (Table 3). The ^{13}C-NMR chemical shift of Me-19 was observed at δ 21.5. The β–positioned and axial Me-20 appeared at δ 19.1, while β–positioned and equatorial Me-17 resonated at δ 16.6. These values revealed the *trans* configuration at the A/B ring junction of **2**. It also showed the downfield shift of the C-2 signal at (δ 69.91) due to hydroxyl group.

The position of hydroxyl group at C-2 was confirmed by HMBC as H-3 (δ 6.11) showed ^2J correlations with C-4 (δ 140.2) and C-2 (δ 69.91) and ^3J correlations to C-18 (δ 170.0) and C-5 (δ 39.1). The coupling constant of H-2 indicated the β and equatorial orientation of the hydroxyl group. The attachment of alkyl chain at C-9 position was also confirmed by HMBC; the AB pattern centered at δ 1.66 and δ 1.57 could be assigned to CH_2-11 which showed ^2J correlations to C-9 (δ 39.6) and C-12 (δ 19.2) and ^3J to C-8 (δ 37.4), C-10 (δ 46.4), and C-20 (δ 19.1) (Fig. 5).

A series of nuclear *Overhauser* effect (NOE) experiments were also carried for **2**, which showed clear difference between Me-19 and Me-20 as well as Me-17 and Me-20, consistent with *cis* relationship between these methyl groups. These results and the fact the irradiation of H-10 did not cause any increase in the intensities of either 19-or 20-methyl signals confirmed the *trans* configuration of the A and B rings of the decalin systems of **2**. No NOE was observed between the H-2 and both CH_3-19 and CH_3-20 indicated their *trans* relationship, establishing the β–configuration and equatorial confirmation of hydroxyl at C-2. Further confirmation of structure **2** was provided by NOEs established the spatial proximity of H-2 and H-10. The structure of **2** was therefore assigned as 2β-hydroxy-15, 16-epoxy-5β, 8β, 9β, 10α-celoda-3, 13(16), 14-trien-18-oic acid (Fig. 6).

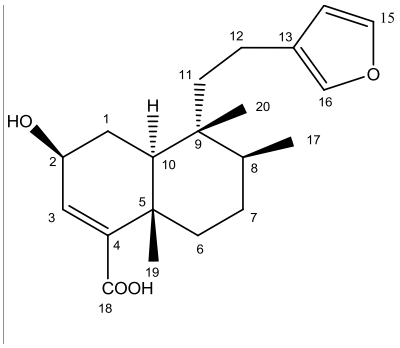
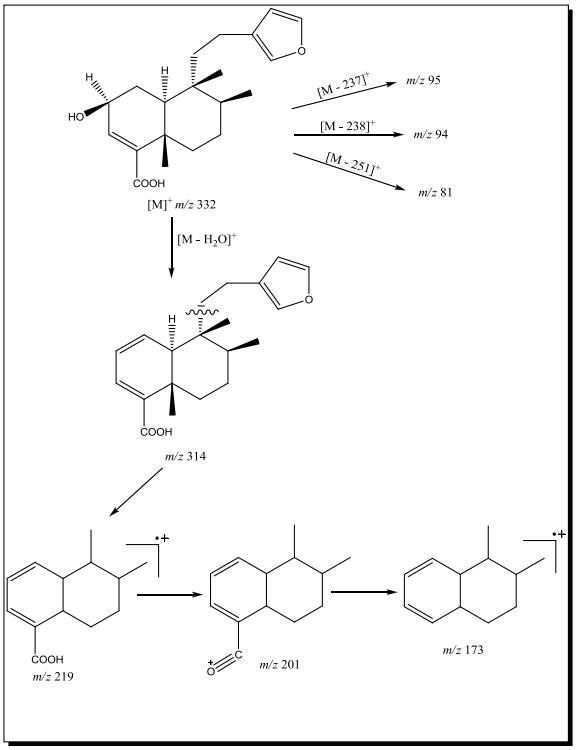


Fig. 4: Structure of compound **2**.



Scheme-2: Mass fragmentation pattern of **2**.

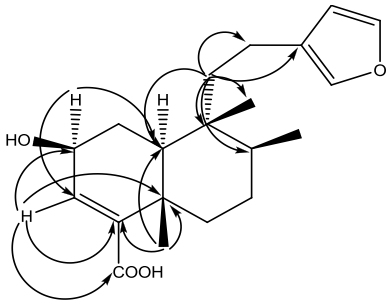
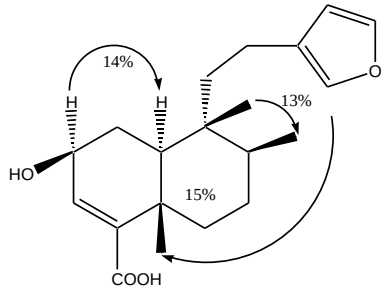


Fig. 5: Important HMBC correlations of **2**.



NOE Interactions of **2**

Fig. 6: Important NOE interactions of **2**.

Table-3: ^{13}C-NMR spectral data of **2**.

Carbon No.	(δ) C-MR (2)	Multiplicity	Carbon No.	(δ) C-NMR (2)	Multiplicity
1	29.0	CH_2	11	36.8	CH_2
2	69.9	CH	12	19.2	CH_2
3	133.5	CH	13	126.8	-C-
4	140.2	-C-	14	111.9	CH
5	39.1	-C-	15	143.9	CH
6	37.2	CH_2	16	139.6	CH
7	28.3	CH_2	Me-17	16.6	CH_3
8	37.4	CH	18	170.0	-C-
9	39.6	-C-	Me-19	21.5	CH_3
10	46.5	CH	Me-20	19.1	CH_3

Table-4: One bond HMQC interactions of **2**

Positions	^{1}H-NMR (δ)	^{13}C-NMR (δ)
1	2.1 (m) 1.43 (m)	29.0
2	4.30 (m)	69.9
3	7.01 (dd, *J* = 3, 4.7 Hz)	133.5
4	--	140.2
5	--	39.1
6	1.17 (m) 2.32 (m)	37.2
7	1.43 (m) 1.50 (m)	28.3
8	1.59 (m)	37.4
9	--	39.6
10	1.38 (br d, *J* = 12.0 Hz)	46.5
11	1.57 (ddd, *J* = 14.4, 12.4, 5.0 Hz) 1.66 (ddd, *J* = 14.4, 12.4, 5.2 Hz)	36.8
12	2.19 (ddd, *J* = 14.4, 12.4, 5.2 Hz) 2.32 (ddd, *J* = 14.4, 12.9, 5.0 Hz)	19.2
13	--	126.8
14	6.26 (dd, *J* = 0.8, 1.7 Hz)	111.9
15	7.32 (t, *J* = 1.7 Hz)	143.9
16	7.2 (br s)	139.6
Me-17	0.82 (d, *J* = 6.5 Hz)	16.6
18	--	170.0
Me-19	1.22 (s)	21.5
Me-20	0.77 (s)	19.1

The HRMS of compounds **3**, isolated from chloroform fraction showed molecular ion peak at *m/z* 330.0785 corresponding to the molecular formula as $C_{17}H_{14}O_7$ (calcd. for $C_{17}H_{14}O_7$ 330.0739). The UV spectrum displayed absorption maximum at 271 nm and 341 nm. These values indicated that compound **3** was a 3-hydroxyflavone. The IR spectrum showed bands at 3310 (OH), 1642 (*α*,*β*-unsaturated ketone), 1603 (aromatic).

^{1}H-NMR spectrum of compound **3** revealed the presence of two methyl singlets at *δ* 3.75 and 3.92 ppm. Similarly another singlet at *δ* 6.69 was due to H-8 proton. The doublets at *δ* 8.12, 6.95, showing only ortho-coupling (1H, *J* = 8.9 Hz) were due to H-2′, 6′ and H-3′,5′ respectively.

^{13}C-NMR spectrum of compound **3** shows that three signals at *δ* 158.9 (C-2), 136.5 (C-3), 175.9 (C=O) confirms that **3** was a 3-substituted flavone. The chemical shift of C-5 (*δ* 152.5) supported the presence of a phenolic group at this position.

The spectral data of compound **3** was in complete agreement to those of reported date [17] and thus compound **3** is identified as 3,5,7-trihydroxy 6,4′-dimethoxyflavanone.

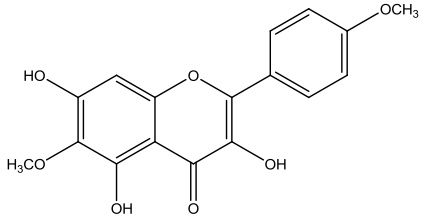


Fig. 7: Structure of compound **3**.

Compound **4** is isolated from ethyl acetate soluble portion showed UV absorption λ_{max} 369 and 266 nm characteristics of flavonoid. The IR spectrum of **4** displayed absorption of bands at 3270 and 1640 cm^{-1} due to the hydroxyl and ketonic functions respectively. The FD-MS showed the molecular ion peak at *m/z* 330, which was further confirmed from HR-EIMS as $C_{17}H_{14}O_7$ (calcd. for $C_{17}H_{14}O_7$ 330.0739).

^{1}H-NMR of compound **4** showed the presence of two methoxy signals and five protons in the aromatic region. Out of the five aromatic protons, the one which appeared as a singlet at *δ* 6.97 was typical 5,6,7-oxygenated substitution pattern, and other four protons showed signals at *δ* 7.08 (2H, d, *J* = 8.9) and 8.35 (2H, d, *J* = 8.9 Hz), confirmed that one methoxy group is at C-4′ position. There were

downfield singlets at δ 12.60 and 10.51 due to hydroxyl groups at C-5 and C-3.

In the mass spectrum peaks at *m/z* 330, 315, 312, for $[M]^+$, $[M\text{-}Me]^+$ and $[M\text{-}H_2O]^+$ appeared with relative intensities of 100, 30 and 6 %. The appearance of $[A_1]^+$ peak at *m/z* 196 also confirmed the two methoxy and one hydroxyl group on ring A. The observation of a $[B_2]^+$ fragment at *m/z* at 121 confirmed the placement one hydroxyl group at C-4′.

The ^{13}C-NMR (BB and DEPT) of compound **4** corroborated the presence of two methyl, five methine and ten quaternary carbons.

The physical and spectral data of **4** was in complete agreement to those reported in literature [18] and thus compound **4** is identified as 3,5, 4′-trihydroxy-6,7-dimethoxyflavone.

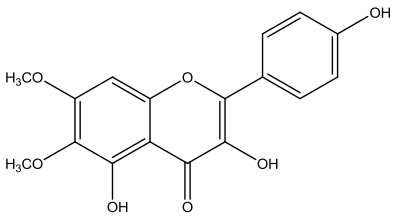


Fig. 8: Structure of compound **4**.

The HR-EIMS of compound **5** showed molecular ion peak at *m/z* 344.0862 corresponding to the molecular formula $C_{18}H_{16}O_7$ (calcd. for $C_{18}H_{16}O_7$ 344.0869). The UV spectrum displayed maxima at 357 and 372 nm. These values indicated that compound **5** was a 3-hydroxyflavone. The IR spectrum showed bands at 3335, 1650, 1600, 1370, and 888 cm^{-1}.

In ^{1}H-NMR spectrum of compound **5** showed a singlet at δ 6.5 corresponding to the H-8 proton while two doublets at δ 7.09 (2H, J = 8.7 Hz) and 8.03 (2H, J = 8.7 Hz) were due to H-2′, H-6′, H-3′, and H-5′, respectively.

The ^{13}C-NMR (BB and DEPT) of compound **5** corroborated the presence of three methyl, five methine and ten quaternary carbons. The signal at δ 94/6 corresponded to C-8, while the signal at 138.1 was due to C-3.The signals at 60.3, 60.5 and 55.9 were due the presence of three methoxyl groups.

By comparison of the literature data [19], compound **5** was identified as 5, 4′-dihydroxy-3, 6, 7-dimethoxyflavone.

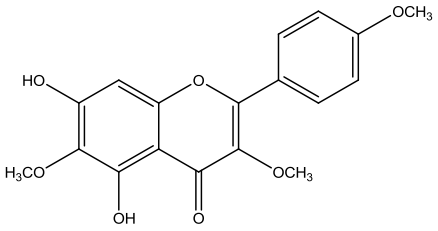


Fig. 9: Structure of compound **5**.

Experimental

General Experimental Conditions

All the solvent used were of commercial grades and doubly distilled before use. In some cases analytical grade solvents (Merck) were also used.

Structure Elucidation

Structures of the isolated and purified compounds were established on the base of spectral data including UV/Visb. (Hitachi U-3200 spectrophotometer), Infrared (IR) spectra (JASCO 302-A Infrared Spectrometer).

^{1}H-NMR spectra were recorded using (Bruker AM-300, AM-400 or AMX-500), ^{13}C-NMR on (Bruker 75, 100 and 125 MHz, respectively) and EI-MS Electron impact (Low resolution) mass spectra (Finnigan MAT 311 and MAT 311 spectrometers, coupled with PDP 11/34 computer system). Peak matching, field desorption (FD) and field ionization (FI) were performed on the Finnigan MAT 312 mass spectrometer. High resolution mass measurements were carried out on Jeol JMS HX 110 mass spectrometer.

Chromatography

All the compounds were Purified using column chromatography (CC) using silica gel (Si 60, 70-230 mesh, E. Merck) as stationary phase and organic solvents as mobile phase.

Flash column chromatography (FCC) was performed on Eyela Flash Chromatograph model EF-10, using silica gel (Si 60, 230-400 mesh, E. Merck) as adsorbent.

Precoated silica gel GF_{-254} preparative plates (20×20, 0.5 mm thick) (E. Merck) were used for preparative thick layer chromatography. Purity of the samples was also checked on the same precoated plates.

Plant Material

Dodonaea viscosa, whole plant material was collected from the hill of Kurram Agency (Pakistan)

and was identified by Dr. Ijaz. Department of Botany, Govt. Post Graduate College Kohat Khyber Pukhtoonkhwa, a voucher specimen was deposited in the same department.

Extraction, Fractionation and Isolation

The plant material was chopped into small pieces, grinded and extracted with MeOH. The methanolic extract was evaporated under reduced pressure which resulted in a gummy material amounting 700 g. The gummy material was partitioned between water and hexane. The thick extract was further shaken out successively with chloroform, ethyl acetate and *n*-butanol.

The chloroform soluble fraction was subjected to column chromatography over silica gel, succeeively eluting with hexane-chloroform, chloroform and chloroform-methanol in increasing order of polarity. The fraction which was eluted with hexane-chloroform (90:10) was subjected to column chromatography and two fractions A and B were obtained of hexane-chloroform. The fraction A with hexane-chloroform as eluent gave a mixture of two major components. Repeated column chromatography using hexane-chloroform (78:22) as an eluent resulted **1** and **3**. The fraction B which was eluted with hexane-chloroform is further chromatograph using hexane: chloroform (70:30) provided **2**. These compounds were characterized through ^{1}H-NMR, mass spectrometry and melting point analysis. The comparison of the obtained data with that of literature revealed us that compound **1**, **2** and **3** are flavonoids.

The ethyl acetate fraction was subjected to column chromatography over silica gel, successively eluting with n-hexane, n-hexane-ethylacetate, ethyl-acetate and ethyl acetate-methanol in increasing order of polarity. The fraction A which was eluted with n-hexane: ethyl-acetate was subjected to column chromatography using various mixture of n-hexane: ethyl acetate fractions. The fraction with n-hexane: ethyl acetate (35:65), as eluent gave a mixture of two major components. Repeated column chromatogram-phy using n-hexane: ethyl-acetate solvent system give us compounds **4** (30:70), and **5** (25:75).

Acknowledgments

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