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Secondary Metabolites from *Jatropha Podagrica* Hook

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Abstract: *The stem bark of* Jatropha podagrica *Hook (Family - Euphorbiaceae) was subjected to a thorough phytochemical investigation. Repeated chromatographic separation and purification of the crude methanol extract of this bark provided six compounds: fraxidin (1), fraxetin (2), scoparone (3), 3-acetylaleuritolic acid (4), β-sitosterol (5) and sitosterone (6). The structures of the isolated compounds were determined by spectroscopic analysis, most notably 2D NMR techniques, and by comparison of their spectral data with previously reported values.*

Keywords: *Jatropha podagrica*, Euphorbiaceae, coumarin, terpenoid

1. INTRODUCTION

Natural products have contributed extensively to the development of new drugs.[1] Therefore, considerable importance has been placed on investigating medicinal plants and herbs for bioactive compounds.[2] A large number of plants have been screened for natural antioxidants, antibiotics and cytotoxic agents to combat various pathological events.[3–5] Expanding on this research, *Jatropha podagrica* was studied here to characterise its secondary metabolites for forthcoming bioactivity study.

Jatropha podagrica Hook (common names: coral nut, Guatemala rhubarb, physic nut; Family - *Euphorbiaceae*) is a shrub native to tropical America, but it is also found in Australia, the Hawaiian Islands, Southern Africa, Mozambique, Zambia and warmer parts of Asia. The genus *Jatropha* is one of the important sources for biologically active phytochemicals. The important biological activities and toxicities of several purified compounds from *Jatropha* species have been well established.[6,7]

Jatropha species are used in traditional medicine for various diseases including skin infections, sexually transmitted diseases such as gonorrhoea,

jaundice and fever.[8–10] Different parts of the plant are also used for antipyretic, diuretic, choleretic and purgative effects.[11] Various medicinal and pesticidal properties, including antimicrobial, antitumour and insect antifeedant activities, have also been attributed to this plant.[12–15] Previous phytochemical investigations of *J. podagrica* led to the isolation of japodic acid, erytrinasinate,[16] *n*-hexacosane, *β*-amyrin, lupeol palmitate, quercetin, apigenin, vitexin, isovitexin, rutin,[17] podacycline A, podacycline B[18] and 3-acetylaleuritolic acid.[19] In the present study, we report the isolation of fraxidin (1), fraxetin (2), scoparone (3), 3-acetylaleuritolic acid (4), *β*-sitosterol (5) and sitosterone (6) from *J. podagrica* Hook.

2. MATERIALS AND METHODS

2.1 Experimental

^{1}H and ^{13}C NMR spectra were acquired using an Ultra Shield Bruker DPX 400 NMR instrument (Bruker BioSpin AG, Fällanden, Switzerland), and the chemical shifts are reported in ppm with respect to TMS or residual non-deuterated solvent signals. The structures of the compounds were identified by spectroscopic analysis and comparison of NMR data with published literature.

2.2 Plant Material

Fresh stem bark of *J. podagrica* was collected from Gazipur, Bangladesh. The tissue was identified by Mr. Sorder Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium, Dhaka. A voucher specimen (DACB Accession no. 31362) was deposited in the Bangladesh National Herbarium, Dhaka, Bangladesh for the collection.

2.3 Extraction and Isolation

Approximately 650 g of the powdered stem bark was soaked in 2.5 l of methanol for 5 days. The whole mixture was then filtered through filter paper, and the filtrate thus obtained was concentrated at 50°C with a rotary evaporator to provide 13.0 g of crude extract. A portion of the methanol extract (11.0 g) was subjected to column chromatography (CC) over silica gel, using gradients of *n*-hexane/dichloromethane followed by dichloromethane and methanol mixtures of increasing polarity to afford a total of 28 fractions (each 100 ml). Column fraction obtained with 5% methanol in dichloromethane was dried and treated with *n*-hexane. After treatment, the fraction yielded a crystal of compound 4 (2.0 mg). The recovered *n*-hexane was evaporated to develop the crystal of compound 6 (2.0 mg). Similar treatment of the column fractions obtained with 2.5%

n-hexane in dichloromethane and 50% dichloromethane in methanol yielded compound 5 (2.0 mg) and compound 2 (15.0 mg), respectively. Column fractions eluted with 15%–50% methanol in dichloromethane were subjected to column chromatography for further purification using gradients of *n*-hexane/ethyl acetate followed by gradients of ethyl acetate and methanol to afford a total of 27 fractions (each 100 ml). Again, *n*-hexane treatment of the column fractions eluted with 40% and 55% ethyl acetate in *n*-hexane afforded compounds 3 (15.0 mg) and 1 (10.0 mg), respectively.

Fraxidin (1): For NMR data see Table 1.

Fraxetin (2): For NMR data see Table 1.

Scoparone (3): ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.37 (1H, d, $J_{3,4}$ = 9.6 Hz, H-3), 7.93 (1H, d, $J_{4,3}$ = 9.6 Hz, H-4), 6.83 (1H, s, H-5), 3.81 (3H, s, 6-OMe), 3.76 (3H, s, 7-OMe); ^{13}C NMR (100 MHz, DMSO-d_6): 160.1 (C-2), 114.6 (C-3), 144.7 (C-4), 100.2 (C-5), 149.7 (C-6), 56.0 (OMe-6), 140.1 (C-7), 60.5 (OMe-7), 138.5 (C-8), 138.3 (C-9), 114.4 (C-10).

3-Acetylaleuritolic acid (4): ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.45 (1H, dd, *J* = 8.0, 4.4 Hz, H-3), 5.52 (1H, dd, *J* = 8.0, 4.4 Hz, H-15), 2.28 (1H, dd, *J* = 14.4, 2.5 Hz, H-18), 0.84 (3H, s, Me-23), 0.87 (3H, s, Me-24), 0.95 (3H, s, Me-25), 0.94 (3H, s, Me-26), 0.92 (3H, s, Me-27), 0.93 (3H, s, Me-29), 0.91 (3H, s, Me-30), 2.03 (3H, s, OAc-3).

β-Sitosterol (5): ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (1H, m, *J* = 7.0 Hz, H-6), 3.50 (1H, m, H-3), 1.00 (3H, s, H_3-19), 0.91 (3H, d, *J* = 6.4 Hz, H_3-21), 0.85 (3H, d, *J* = 6.0 Hz, H_3-29), 0.83 (3H, d, *J* = 6.0 Hz, H_3-26), 0.81 (3H, d, *J* = 6.0 Hz, H_3-27), 0.67 (3H, s, H_3-18).

Sitosterone (6): ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.71 (1H, s, H-4), 1.17 (3H, s, H_3-19), 0.91 (3H, d, *J* = 6.4 Hz, H_3-21), 0.85 (3H, t, *J* = 7.2 Hz, H_3-29), 0.83 (3H, d, *J* = 6.8 Hz, H_3-26), 0.81 (3H, d, *J* = 6.8 Hz, H_3-27), 0.70 (3H, s, H_3-18).

Table 1: NMR spectral data for compounds 1 and 2*.

Position	Compound 1 (in $CDCl_3$/2%CD_3OD)				(in DMSO-d_6)	
			HMBC			
	δ_C	δ_H	^2J	^3J	δ_C	δ_H
2	161.4	–	–	–	160.1	–
3	114.4	6.26 d	161.4(C-2)	114.1(C-10)	114.6	6.37 d
4	144.4	7.60 d	114.4(C-3)	161.4(C-2)	144.7	7.93 d
			114.1(C-10)	138.3(C-9)		
5	99.7	6.44 s	–	140.4(C-7)	100.2	6.83 s
				138.3(C-9)		
6	150.2	–	–	–	149.7	–
6-OCH_3	56.1	3.83 s	–	150.2(C-6)	149.7	3.81 s
7	140.4	–	–	–	56.0	–
7-OH	–	–	–	–	140.1	–
7-OCH_3	61.0	3.91 s	–	140.4(C-7)	60.5	3.76 s
8	138.5	–	–	–	138.5	–
8-OH	–	–	–	–	–	9.85 brs
9	138.3	–	–	–	138.3	–
10	114.1	–	–	–	114.4	–

Position	Compound 2 (in DMSO-d_6)			
			HMBC	
	δ_C	δ_H	^2J	^3J
2	160.5	–	–	–
3	111.8	6.21 d	160.5(C-2)	110.2(C-10)
4	145.0	7.87 d	111.8(C-3)	160.5(C-2)
			110.2(C-10)	100.3(C-5)
				139.3(C-9)
5	100.3	6.78 s	145.3(C-6)	145.0(C-4)
				139.3(C-7)
				139.3(C-9)
6	145.3	–	–	–
6-OCH_3	56.0	3.81 s	–	145.3(C-6)
7	139.3	–	–	–
7-OH	–	9.44 brs	–	–
7-OCH_3	–	–	–	–
8	132.8	–	–	–
8-OH	–	9.44 brs	–	–
9	139.3	–	–	–
10	110.2	–	–	–

*^{13}C and ^1H data acquired at 100 and 400 MHz, respectively.

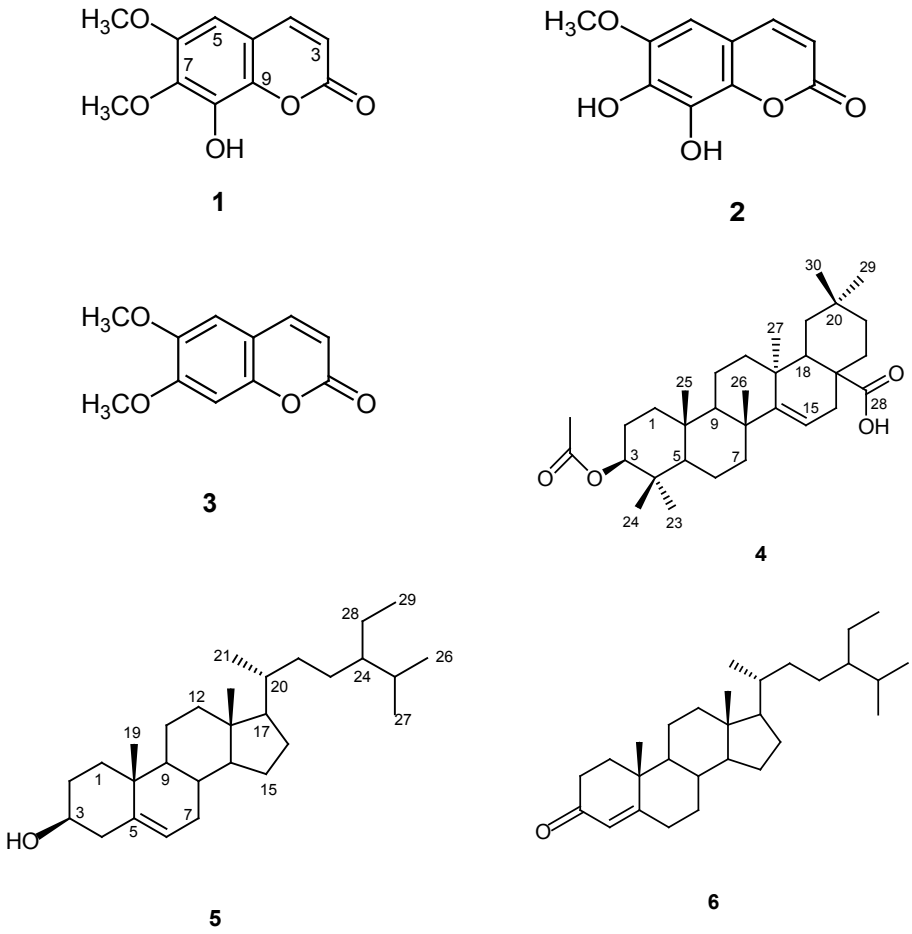

Figure 1: Structures of the isolated compounds (1–6).

3. RESULTS AND DISCUSSION

Extensive chromatographic separation and purification of the methanolic extract of the stem bark of *J. podagrica* provided compounds 1–6.

The ^{13}C NMR spectrum (100 MHz, $CDCl_3$/2% CD_3OD) of compound 1 displayed 11 carbon resonances, while HSQC and DEPT 135 (100 MHz, $CDCl_3$/2% CD_3OD) spectra indicated that 5 out of 11 carbons were attached to protons. The ^1H NMR (400 MHz, $CDCl_3$/2% CD_3OD) and DEPT 135 spectra of compound 1 revealed the presence of three methine carbons and two *O*-methyl groups. The ^1H NMR of compound 1 in $CDCl_3$/2% CD_3OD displayed signals

characteristic of a coumarin. The lactone ring protons showed an AB pattern for H-3 (δ 6.26, d, J = 9.4 Hz) and H-4 (δ 7.60, d, J = 9.4 Hz).[20] The singlet at δ 6.44 ppm could be attributed to the aromatic proton at C-5. The identification of the compound and its ^{13}C assignments were established unambiguously by 2D NMR studies. The ^{1}H and ^{13}C NMR spectra of compound 1 were also recorded in DMSO-d_6 to compare the data with the published values. A new signal as a broad singlet at δ 9.85 was found due to the presence of a phenolic hydroxyl group at C-8. These data for compound 1 were found to be identical to those reported for the compound fraxidin.[19, 21] On this basis, compound 1 was positively identified as fraxidin.

The ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound 2 displayed 10 carbon resonances, while HSQC and DEPT 135 (100 MHz, DMSO-d_6) spectra indicated that 4 out of 10 carbons were attached to protons. The structure of compound 2 was further revealed by direct comparison of its spectral data with those of coumarin 1. It was evident from the ^{1}H and ^{13}C NMR spectra and also from the DEPT 135 experiment that almost all chemical shifts of coumarin 2 were superimposable with those of coumarin 1, except for the presence of one 7-OH group instead of a 7-OCH_3 group. These data for compound 2 were found to be identical to those reported for the compound fraxetin.[21] Thus, compound 2 was identified as fraxetin.

The spectral data of compound 3 were in close agreement with those of 1 and 2. The ^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR spectra (100 MHz, DMSO-d_6) as well as the DEPT 135 experiment suggested the presence of an additional aromatic proton for compound 3 with respect to 1 and 2, and the absence of one hydroxyl group compared to 1. The absence of two hydroxyl groups and the presence of an additional *O*-methyl group compared to 2 were also discovered. The absence of any coupling between the two aromatic protons at C-5 and C-8 necessitated the accommodation of the two *O*-methyl groups at C-6 and C-7. The ^{1}H NMR and ^{13}C NMR data of compound 3 were found to be identical to those reported for scoparone.[22] Taking these data into consideration, compound 3 was identified as scoparone.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound 4 displayed seven singlets at δ 0.84, 0.87, 0.91, 0.92, 0.93, 0.94 and 0.95 ppm, each integrating to three protons and assignable to seven tertiary methyl groups. The singlet at δ 2.03 ppm could be ascribed to an acetoxy group at C-3. A one-proton double-doublet at δ 4.45 ppm (1H, dd, J = 8.0, 4.4 Hz) was possibly the proton on the carbon atom bearing the acetoxy group (C-3). One one-proton double-doublet at δ 2.28 ppm (1H, dd, J = 14.4, 2.5 Hz) was assigned to the methine proton at C-18. A one-proton double-doublet at δ 5.52 ppm (1H, dd, J = 8.0, 4.4

Hz) could be ascribed to the olefinic proton at C-15. All of the coupling constants and splitting patterns indicated the presence of a trisubstituted double bond between the 14 and 15 positions. The structure of compound 4 was thus identified as 3-acetylaleuritolic acid by comparing its ^{1}H NMR data with reported values.[23]

The structures of *β*-sitosterol and sitosterone were confirmed by direct comparison of their spectral data with the reported values.[24, 25]

4. CONCLUSION

Phytochemical investigation of the crude methanol extract of *J. podagrica* led to the isolation of six metabolites: fraxidin (1), fraxetin (2), scoparone (3), 3-acetylaleuritolic acid (4), *β*-sitosterol (5) and sitosterone (6). Further biological study is required to explore their potential therapeutic activities.

5. ACKNOWLEDGEMENTS

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