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Research Article

A New Triterpenoid Saponin and Antimicrobial Activity of Ethanolic Extract from *Sapindus mukorossi* Gaertn.

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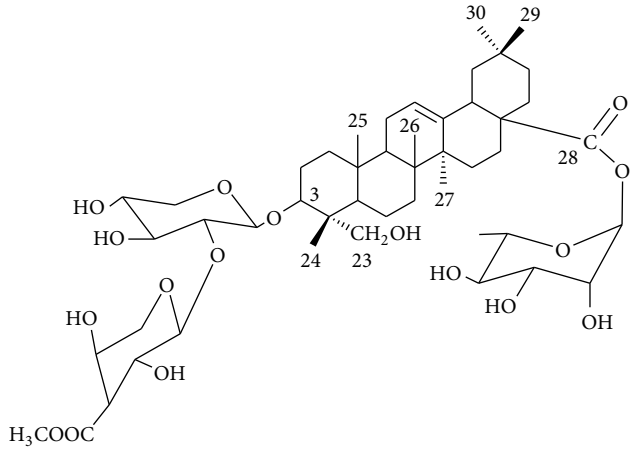
A new acetylated triterpenoid saponin elucidated as hederagenin-3-*O*-β-D-xylopyranosyl $(2 \rightarrow 1)$-[3-*O*-acetyl-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosylester has been isolated from pericarps of *Sapindus mukorossi* Gaertn. The structure of the compound was determined by means of chemical and spectral analysis including advanced 2D NMR studies. The ethanolic extract from pericarps of the plant showed significant *in vitro* antimicrobial activity against various test organisms by Agar well diffusion method.

1. Introduction

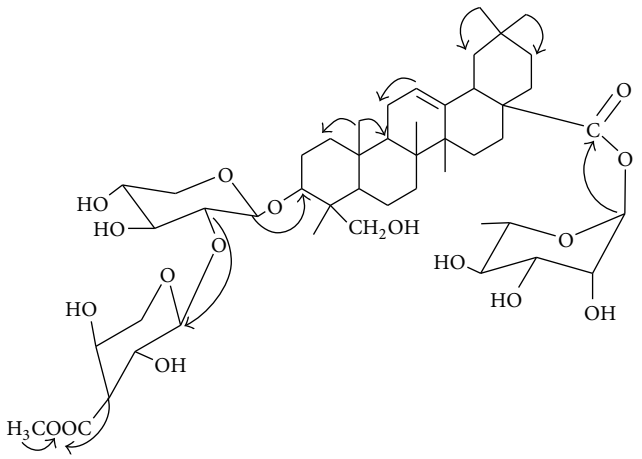
Sapindus mukorossi Gaertn. of family Sapindaceae is well-known soap nut tree, distributed in tropical and subtropical regions of Asia. Fruits are popular ingredient in Ayurvedic shampoos and cleansers. The plant is an important remedy for relieving cough, detoxification, emetic, contraceptive, treatment of excessive salivation, epilepsy, and chlorosis [1–3]. Previous studies on the plants of this genus led to the isolation of triterpenoids, saponins [4–7] fatty acids [8], and flavonoids [9]. The present paper illustrate the isolation and structure elucidation of a new acetylated triterpenoid saponin (**1**) (Figure 1) for the first time from this species.

2. Result and Discussion

Acetylated triterpenoid saponin was isolated from *n*-butanol (BuOH) extract of the pericarps of *S. mukorossi*. The *n*-BuOH extract was subjected to repeated column chromatography and the fractions were eluted with methanol in chloroform. The compound **1** was purified from among number of reported compounds with (50 mg) yield. The compound **1** was crystallized as an amorphous solid. It revealed an $[M + H]^+$ ion peak at *m/z* 925.10684 in HR-ESIMS in agreement with molecular formula $C_{48}H_{76}O_{17}$ and melting point is 202–206°C. The IR spectrum exhibited absorptions at 3450 (OH) and 1731 (C=O of ester) cm^{-1}. Positive results with Liebermann-Burchard and Molisch's reagents make obvious its triterpenoidal glycosidic nature. ^{13}C NMR and DEPT spectra exhibited 48 carbon signals for eight methyl, thirteen methylene, ninteen methane, and eight quaternary carbons. Acid hydrolysis of **1** afforded triterpenoid as the aglycone, D-glucuse, D-xylose, and L-rhamnose, respectively. The sugar moieties were established by the comparison of spectral data from previously reported compounds isolated from this plant and also confirmed by authentic samples, respectively [10, 11]. The downfield ^{13}C-NMR chemical shift at δ C 81.5 and the upfield ^{13}C-NMR chemical shift at δ C 178.8 suggested that **1** was a triterpenoid saponin with glycosidic linkages at C-3 through an ether bond and at C-28 through an ester bond. The anomeric proton signals were at δ H 5.16 (d, 7.8), 5.05 (d, 7.0), and 4.80 (d, 9.2) with the corresponding anomeric carbon signals at δ 104.8, 106.5, and 94.8 according to HMQC and HMBC spectrum, respectively. The linkage of the sugar units at C-3 of the aglycone was established from the following HMBC correlations: H-1 (δ_H 5.1) of xylose



(a)



(b)

Figure 1: ^{1}H-^{13}C HMBC correlation.

with C-3 (δ_C 81.5), H-1 (δ_H 5.0) of arabinose with C-2 (δ_C 72.2) of xylose, and H-1 of rhamnose (δ_H 4.8) with C-28 (δ_C 178.8) of the aglycone as shown in Table 1. Since the chemical shift of C-3 of aglycone is linked to the H-1 of xylose was same as that C-2 of xylose was linked to the H-1 of arabinose and C-28 was directed linked to the H-1 of rhamnose, their linkages of sugars could be finally determined from the NOE experiment. The cross-peak between the H-1 (δ_H 5.1) of xylose and H-1 (δ_H 5.1) of arabinose indicated that the xylose moiety of C-2 was linked to C-1 of arabinose, respectively. A broad signal at δ 5.24 (H-12) in the ^{1}H NMR is due to the presence of an olefenic proton which was further supported by ^{13}C NMR values at δ 123.60 (C-12) and 145.02 (C-13). In the ^{13}C NMR spectrum, the values at δ 64.2 (C-23) and 81.53 (C-3) were corroborated to a methylene carbon linked to hydroxyl group and a tertiary carbon linked to sugar moiety, respectively. Moreover, a downfield signal at δ 178.8 (C-28) was clearly indicative to a carbonyl carbon [12]. The index of hydrogen deficiency for $C_{48}H_{76}O_{17}$ (= 11) showed the presence of eight rings (pentacyclic rings and three sugar rings) and three double bonds (an olefenic and two carbonyl). The ^{1}H NMR value at δ 2.15 and ^{13}C NMR signals at δ 173.48 (−COO) and 22.05 (Me) indicated the presence of acetoxyl group attached to arabinose ring as found in the previous literature [11]. From above discussion, it is quite obvious that two sugar moieties (xyl → ara) were linked to C-3 and the third (rham) was attached to C-28 of pentacyclic triterpenoidal skeleton. All protons and carbons of each sugar unit were assigned on the basis of extensive NMR experiments including HMQC, COSY, NOE, and HMBC. The LCMS of **1** was found very informative in support of the structural confirmation which showed a quasimolecular ion peak at *m/z* 925 (100%) along with other fragment ions at *m/z* 882, 750, 618, and 471 due to the loss of sugar fragments. Hence, compound **1** was elucidated ashederagenin-3-*O*-β-D-xylopyranosyl (2 → 1)-[3-*O*-acetyl-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosylester.

Antimicrobial activity of ethanolic extract from pericarps of *S. mukorossi* was determined by the agar well diffusion method. The extract showed significant inhibitory activity against *Esherichia faecalis*, *Pseudomonas aeuroginosa*, *Staphylococcus aureus*, *Alcaligenes denitrificans*, *Klabsiella pneumoniae*, *Bacillus cereus*, *Pseudomonas alcaligenes*, *Micrococcus luteus*, and *Bacillus subtillis*. The ethanolic extract showed most significant activity against *Pseudomonas aeruginosa*, *Esherichia faecalis*, and *Bacillus cereus* by inhibition zone diameter (IZD) value of 20, 19, and 17 mm, respectively, at minimum inhibitory concentration (MIC) of 100 μg/mL, whereas the IZD values of 15, 17, and 15 mm, respectively, were shown at MIC value of 50 μg/mL compared with positive control.

3. Experimental

3.1. General. Melting point was recorded on the Perfit melting point apparatus. IR spectra were recorded on Perkin-Elmer, spectrum RX I FT-IR spectrometer (KBr discs). NMR spectra were obtained on Bruker, 400, Ultra shield NMR spectrometer (300 MHz for ^{1}H and 125 MHz for ^{13}C, DMSO-d_6 as solvent, and TMS as internal standard). MS were recorded on LCMS Q-TOF Micro mass spectrometer and LCMS-LCQ, Finnigam, MAT mass spectrometer. Column chromatography was performed on silica gel (Merck 60–120 mesh, 15 × 100 cm). Thin layer chromatography was carried out on silica gel (Merck 10–40 μm) precoated plates which were visualised by spraying with 7% H_2SO_4 as a universal spray reagent.

3.2. Plant Material. Fresh pericarps (6 kg) of *S. mukorossi* were collected from Durgadhar, District Rudrapryag (Uttarakhand) during March 2009 and identified by Taxonomical Laboratory, Department of Botany, H.N.B. Garhwal University, Srinagar India. A voucher specimen (GUH-8644) of the plant was deposited in Departmental Herbarium for future records.

3.3. Extraction and Isolation. Shade-dried and powdered pericarps (4 kg) were extracted with 95% ethanol (5 L, 3

Table 1: ^{13}C, ^{1}H and HMBC data of compound **1** in DMSO d^6.

Position	Aglycone δ_C ppm	δ_H ppm	HMBC	Position	Glycone δ_C ppm	δ_H ppm	HMBC
1	38.05	1.36		Xyl			
2	22.12	1.98 m		1′	104.83	5.16 (d,7.8)	
3	81.53	2.73 (dd, 11.2, 4.0)	C-1′	2′	72.20	4.06 (d,9.2)	C-1″
4	36.03	—		3′	77.95	3.84 m	
5	15.55			4′	68.07	4.34 m	
6	26.13	—		5′	70.03	3.38 (d, 11.2)	
7	33.15	—					
8	41.02	—		Ara			
9	47.05	—		1″	106.54	5.05 (d, 7.8)	
10	42.53	—		2″	76.22	3.14 m	
11	27.82	—		3″	72.23	4.56 (d, 9.2)	
12	123.60	5.24 m	C-11, 13	4″	69.53	3.98 m	
13	145.02	—		5″	65.72	4.62 (dd, 9.2, 3.3)	
14	45.48	—					
15	26.13	—					
16	22.34	—		Rha			
17	46.23	—		1‴	99.85	4.80 (d, 9.2)	C-28
18	41.63	—		2‴	71.22	4.73 m	
19	45.52	—		3‴	79.53	3.38 m	
20	30.34	—		4‴	73.82	3.18 (d, 11.2)	
21	33.91	—		5‴	64.52	4.2 (d, 9.2)	
22	35.31	—		CH_3	17.52	1.24 (d, 6.2)	CO
23	64.22	3.34, 3.51		CO	173.48	—	
24	12.83	1.09		CH_3	22.05	2.15 s	CO
25	16.77	1.09	C-1, 8				
26	17.35	1.09					
27	25.72	0.70					
28	178.83	—					
29	32.92	0.87	C-21				
30	24.54	0.87	C-19				

Table 2: IZD values (in mm) of ethanolic extract from pericarps of *S. mukorossi*.

Test organism	Ethanol extract			Kanamycin
	0 μg/mL	50 μg/mL	100 μg/mL	100 μg/mL
E. faecalis	NZ	17	19	25 mm
P. aeuroginosa	NZ	15	20	23 mm
S. aureus	NZ	12	14	20 mm
A. denitrificans	NZ	11	14	22 mm
K. pneumoniae	NZ	10	11	25 mm
B. cereus	NZ	15	17	26 mm
P. alcaligenes	NZ	10	12	25 mm
M. luteus	NZ	11	12	23 mm
B. subtilis	NZ	9	14	22 mm

NZ: no zone of inhibition.

times) at 35°C on a heating mantle. The extraction mixture was filtered off and concentrated under reduced pressure to yield black brown residue (380 g). This residue was fractionated with ethyl acetate (EtOAc), yielded EtOAc soluble and insoluble fractions. Insoluble part (190 g) was partitioned with *n*-butanol and water. The crude extract (110 g) obtained from the butanolic layer was subjected to column chromatography using silica gel (Merck 60–120 mesh, 500 g) with solvent gradient system in order of increasing polarity. The elution was first started with $CHCl_3$: MeOH

[19 : 1] by increasing polarity of MeOH [19 : 1 → 7 : 3]. The fractions were collected at every 50 mL and the elution with $CHCl_3$: MeOH (22 : 3) afforded compound **1**.

3.4. Acid Hydrolysis. Compounds **1** (3 mg) in 10% HCl-dioxane (1 : 1, 1 mL) was refluxed at 80°C for 4 h in a water bath, neutralized with aqueous Ag_2CO_3, filtered, and then extracted with $CHCl_3$ (1 mL × 3). Aqueous layer (monosaccharide portion) was examined by TLC with *n*-BuOH-AcOH-H_2O (4 : 1 : 5, upper layer) and compared with authentic samples of D-glucose, D-xylose, and L-rhamnose.

4. Antimicrobial Assay

Evaluation of antimicrobial activity of ethanolic extract was carried out by the agar well diffusion method [13, 14]. The extract was reconstituted in 20% DMSO for the bioassay analysis [15]. Different culture media were bioassayed for inhibition zone diameter (IZD) and minimum inhibitory concentration (MIC) determination using Kanamycin as a positive control. In this method, pure isolate of each microbe was subcultured on the recommended specific media for each microorganism at 37°C for 24 hrs. One hundred microlitre (100 μL) of inoculum of each test organism was spread onto the specific media plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells or cups of 8 mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24 h [16–18] and the zone of inhibitions was measured. MIC was determined as the least concentration of extract inhibiting the growth of the test organisms during 24 h. The results of antimicrobial activity are shown in Table 2.

5. Concluding Remarks

The present study on *S. mukorossi* which belongs to family Sapindacea, isolated a new triterpenoid saponin hederagenin-3-*O*-β-D-xylopyranosyl (2 → 1)-[3-*O*-acetyl-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosylester which was compared to the previous reported triterpenoid saponins. It is new due to the attachment of rhamnose at C-28 position which has not been reported earlier in the previous literature. Consequently, this compound is a novel acetylated triterpenoid saponin. Further the ethanolic extract of *S. mukorossi* showed significant activity against the test organism as compared with positive control. The biological potential for the compound has to be further investigated.

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