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Isolation and Characterization of 3-O-Glucopyranosyl oleanane-12,15-diene from hexane extract of *Mesua ferrea* Linn. (Seeds)

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Abstract
Mesua ferrea Linn. Has been investigated by many workers for its constituents. Previous phytochemical studies have revealed this genus to be rich in secondary metabolites including phenylcoumarins, xanthones and triterpenoids. So far, not many studies have been carried out on this genus but there are some reports on this plant. Our recent study on the hexane extract of the seeds of Mesua ferrea Linn, have led to the isolation of steroidal compound. The structure of the compound has been established by modern spectroscopic techniques such as IR, ^{1}H-NMR, ^{13}C-NMR and mass-spectroscopy and identified as 3-O-Glucopyranosyl oleanane-12,15-diene.

Keywords: *Mesua ferrea* L., medicinal plant, 3-O-glucopyranosyl oleanane-12,15-diene

Introduction
M. ferrea L. is a tree of tropical Asia and belongs to family Guttiferae. Various parts of the plant are used medicinally in India, China, Malaysia and Thailand [1]. Its bark is given in treatment of cough, dysentery, vomiting, sore throat and fever. Their flowers are astringent and stomachic [2]. The leaves and flowers in combination with other drugs are used for the treatment of snake bite and scorpion sting. The seed oil is used as an embrocation in rheumatism and found useful in the treatment of itch [3]. Our ongoing research is focused on the screening of the extracts of *M. ferrea* L. (Seeds) on the screening promising bioactivities which can then be developed into drugs through preclinical and clinical developments.

Materials and methods
Chemicals: The organic solvents used in the experiments were of analytical grade and purchased from Qualigen Chemicals, India. The other chemicals used were of analytical grade and obtained from Merck, India.

Plant sample: Air-dried seeds of *M. ferrea* L. were collected from the local spice market of District Ujjain, India. Plant samples were duly authenticated by department of Botany, Vikram University, Ujjain, India.

Extraction procedure
The plant samples were washed several times with tap water and finally with distilled water to remove dust. The samples were dried under shade at room temperature. The seeds were separated from dried pods by crumbling and then screening. The shade dried seeds were further ground by means of a mechanical blender (Bajaj GX10, India) to fine powder. One hundred grams of the seed powder was sequentially extracted for 3 days with each solvent hexane (500 mL ×3) and ethanol (500 mL×3) using a Soxhlet apparatus over a water bath. The extracts obtained were filtered through Whatman No. 1filter paper and then evaporated to dryness by using a rotary evaporator (Buchi, Switzerland). The final crude extracts were collected in an airtight container and then refrigerated at 4 ± 2◦C until further use.

Processing of the Hexane extract
The extract showed the presence of several compounds on TLC examination. This extract was fractionated on Silica gel G (Table No.1). The column was eluted with various solvents in their increasing order of polarity. Benzene fractions (Fr. No. 44-51) of this column yielded crystalline compound in pure form designated as SD6.The compounds was identified using IR, ^{1}HNMR, ^{13}CNMR and Mass spectroscopy.

Column chromatography of hexane extract

Table 1: Wt. of extract 100gm, Wt. of silica gel G- 1000gm

Fraction No.	Eluent	Ratio(v/v)	Volume collected(ml)	Spots on TLC
1-10	Hexane	-	2500	2 spots
11-18	Hexane : Benzene	9:1	2000	3 spots
19-27	Hexane : Benzene	3:1	2000	4 spots
28-34	Hexane : Benzene	1:1	1500	2 spots
35-43	Hexane : Benzene	1:3	2000	3 spots
44-51	Benzene	-	2000	2 spots
52-64	Ethyl acetate	-	3000	4 spots

65-74	Benzene : Methanol	8:2	1750	3 spots
75-85	Benzene : Methanol	1:1	2500	3 spots
86-94	Benzene : Methanol	1:3	2000	2 spots
95-104	Methanol	-	2500	4 spots

Processing of Benzene pure (1:1, v/v) Fraction No. 44-51

Table 2: Wt. of sample- 6gm, wt. of silica gel G- 1000gm

Fraction No.	Eluent	Ratio (v/v)	Volume collected (ml)	Spots on TLC	Compound code
1-4	Hexane	-	800	2 spots	
5-8	Benzene	-	800	3 spots	
9-12	Benzene : Ether	3:1	800	2 spot	
13-16	Benzene : Ether	1:1	600	2 spots	
17-20	Benzene: Ether	1:3	800	2 spots	
21-24	Benzene : Methanol	1:1	800	1 spot	SD6
25-28	Methanol	pure	800	2 spot	

Results and Discussion

Characterization of compound SD6

The Benzene : Methanol (1:1) fraction showed the presence of a single compound on TLC examination. All the similar fractions were mixed together and solvent was removed to yield a solid mass, which was recrystallized from chloroform and methanol, yielded pure compound designated as SD6. On the basis of elemental analysis and mass spectrum its molecular formula was found to be $C_{30}H_{52}O$, M^+ 428.

IR Spectrum (ʎmax, KBr, cm^{-1})

The absorption band at 3434 cm^{-1} was due to hydroxyl group and band at 2967 cm^{-1} was due to aliphatic stretching vibration mode. Band at 1384 cm^{-1} was due to bending vibration. Band at 1678cm^{-1} was due to C=C group. Bands at 1165, 1051, 929 cm^{-1} were due to C-O-C linkage [15-16].

^{1}HNMR spectrum (300MHz, Pyr, TMS,δ)

The ^{1}HNMR spectrum closely revealed the molecule to be of terpenoid type. The complex signals in δ 3.9-5.2 region indicated the molecule to be glycosylated. The olefinic proton of the double bond resonated as a doublet at δ 5.37 and its position was confirmed at C-12 and C-15 by comparison of data from literature. The assignments of methyls were done after through study of the literature related with the proposed structure [4-12]. The intense singlet at δ 1.28 was attributed to methyls at C-28 and C-29. The doublets centered at δ 0.88, δ 0.91, δ 0.89 appeared for the methyls at C-21, C-28 and C-29 respectively.

The protons of sugar resonated at

1. δ 4.56 d (J=8.2)
2. δ4.79 t (J=8.1)
3. δ5.20 t (J=9.5)
4. δ5.05 t (J=9.3)
5. δ 3.99 ddd (J=2.5, 4.8, 10.0 Hz)
6. δ 4.09 dd (J=2.3, 12.2 Hz)
   δ 4.26 dd (J=4.8, 12.2 Hz)

The sugar was identified to be glucose by comparison of the data with the literature. A multiplet centered at δ 3.99 further confirmed the position of glycosidation at C-3 in the molecule. The ^{13}CNMR and mass spectrum provided further evidences in support of the proposed structure [13-14].

Mass spectrum

The mass spectrum showed M^+ 428 and the molecular formula was found to be $C_{30}H_{52}O$ for the aglycone moiety. The bond fission at C12-C13 and C8-C14 yields intense fragments of mass m/z 204 and 208. Relatively weak abundant ion peak at m/z 288 was obtained from the loss of methyl group and the entire side chain from the molecule.

SD6 was thus identified as 3-O-Glucopyranosyl oleanane-12,15-diene.

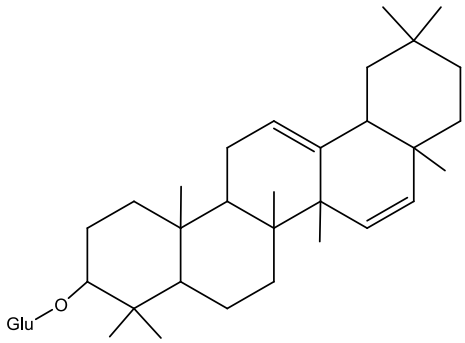

Fig 1: 3-O-Glucopyranosyl oleanane-12,15-diene

Identification of Compound SD6

Designation: 3-O-Glucopyranosyl oleanane-12, 15-diene.
Molecular formula: $C_{30}H_{52}O$
Solubility: Pyridine
Molecular ion peak: M^+ 428
State: solid

IR Spectrum (ʎmax, KBr, cm^{-1})

3434, 2967, 1384, 1678, 1165, 1051, 929 cm^{-1}

^{1}HNMR spectrum (300MHz, Pyr, TMS,δ)

δ 5.37 (d, J=5.7, C-12, -C=CH), δ 3.99 (m, J=10.4 Hz, 1H, C-3), δ 1.9-2.1 (bunch of s, sugar OAc), δ 4.60 (d, 1H, J=8.0 Hz, anomeric proton), δ o.68 (s, 3H, C-18 Me), δ 1.28 (s, 6H, C-28 and C-29 Me).

^{13}C NMR spectrum (75 Hz, Pyr, ppm)

Details are given in table.

ESI-MS Spectrum (m/z, rel. inte.)
[M^+] 428, 288, 204,208

Table 3: ^{13}CNMR chemical shift values of SD6

Carbon NO.	C	CH	CH_2	CH_3
1			37.4	
2			23.4	
3		79.09		
4	39.8			
5		50.8		
6			19.5	
7			32.5	
8	37.4			
9		50.8		
10	36.9			
11			23.4	
12	122.4			
13	141.4			
14	29.05			
15		129.9		
16		135.3		
17	51.9			
18		39.8		
19			38.0	
20	30.7			
21			36.9	
22			29.0	
23				19.5
24				19.1
25				19.9
26				24.3
27				26.2
28				14.7
29				32.5
30				21.9

Conclusion
The results of the present investigation showed the occurrence of 3-O-Glucopyranosyl oleanane-12,15-diene.type compound in plant kingdom. The title Compound was isolated from this plant for the first time.

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