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S Prabhavathi
PG & Research Department of Chemistry, Government Arts College (Autonomous), Coimbatore - 641018, Tamil Nadu, India.

S Vijayalakshmi
PG & Research Department of Chemistry, Government Arts College (Autonomous), Coimbatore - 641018, Tamil Nadu, India.

Correspondence:
S Vijayalakshmi
PG & Research Department of Chemistry, Government Arts College (Autonomous), Coimbatore - 641018, Tamil Nadu, India.

Identification of new aliphatic hydroxy ether from the root bark of *Ziziphus oenoplia*

S Prabhavathi, S Vijayalakshmi

Abstract
Out of three solid compounds separated from the chloroform extract of the root bark of *Ziziphus Oenoplia*, recently one was identified as 7a-hydroxy-6-isopropenyl-3,3,3a,5,6a,10,10-11b,octamethyl-hexadecahydro benzo [de]anthracene5-carboxylic acid. On continuation of our interest, the second compound was identified as an acyclic hydroxy ether from IR,^{1}H,^{13}C, ^{1}H-^{1}H COSY,^{1}H-^{13}C HETCOR, 135 DEPT, HMBC, and mass spectral data. Further the antimicrobial studies on the new compound also been done on various stains of pathogenic bacteria and fungi in comparison with the standard.

Keywords: *Ziziphus oenoplia*, Root bark, new hydroxy ether, antimicrobial studies.

1. Introduction
Ziziphus oenoplia (*L*) Mill (Family Rhamnaceae), commonly known as Jackal Jujube, small fruited Jujube or wild Jujube is a flowering plant with a broad distribution through tropical and subtropical Asia and Australia. It is a spreading, sometimes climbing, thorny shrub growing to 1.5 m in height [2, 3].
Chemically the root bark of *Ziziphus oenoplia* contains cyclopeptide alkaloids Zizyphine A, Zizyphine B, Betulinicacid, d-glucose, d-fructose, sucrose and unidentified Polysaccharides [4, 5].
Considering the above facts and the biological importance of the root, our interest turned to identify the second compound separated from chloroform extract of the root bark of *Ziziphus oenoplia*.

2. Materials and Methods
The roots of *Z. oenoplia* were collected in and around Coimbatore district and were identified at BSI, Coimbatore, and Tamil Nadu. The collected roots were washed under running tap water and cut into small pieces of 2-3 cm, shade dried (30 ºC, 45% relative humidity) for 15 days and then grinded to get a coarse powder. The powder was stored in air tight container for further use.

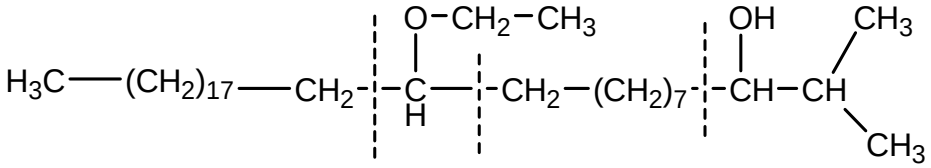
3. Experimental
Melting point was determined by open capillaries and are uncorrected. The purity of the compound was checked by TLC using silica gel and suitable solvent system. IR spectra was recorded on Perkin-Elmer 1000 spectrophotometer in KBr. The^{1}H and ^{13}C NMR spectra was recorded on a Bruker DRX-400 NMR spectrophotometer using TMS as internal standard and chemical shifts are expressed in δ ppm. Mass spectra was recorded on a GC-LC/MS 5970 mass spectrophotometer. Elemental analysis was performed on Varian-EL III CHNOS-Elemental analyser. Analysis results were within 0.4% of the calculated value.

4. Results and Discussion
The chloroform extract of the root bark of *Ziziphus oenoplia* on further column chromatography yielded the second compound as white crystalline powder. It's molecular formula $C_{34}H_{70}O_2$ (M.W: 510) was arrived from mass spectra and analytical data.
Usual tests showed that it was an aliphatic saturated compound. The IR $_{KBr}$ spectrum indicated the presence of hydroxy at γ_{max} 3486 cm^{-1} and ether groups at γ_{max} 1158 cm^{-1} respectively. The ^{1}H and ^{13}C NMR spectra showed signals only in the aliphatic region. In ^{1}H-NMR spectra it was observed 69 H-atom to be present in the aliphatic region and one C-OH signal. The presence of isopropyl group was confirmed from the one proton septet at δ 3.49-3.54 ppm and six proton doublet at δ 4.98-5.18 ppm respectively.

^{13}C NMR (DMSO- d^6) showed 34 carbon signals and analysis of ^1H –^1H COSY and^1H-^{13}C HSQC, HMBC led to the assignment of the spin systems for hydroxyl group and suggested that it was an aliphatic compound with a long straight chain. From DEPT 135 spectra, 7odd carbons at ∂ 55.30, ∂55.34, ∂50.38, ∂48.99, ∂38.05, ∂28.56, ∂19.40, gave positive signal. The two signals at ∂110.56 and ∂162.85 corresponds to OC_2H_5 and -OH (D_2O exchangeable) groups respectively. The presence of ether was in addition confirmed from^1H NMR andDEPT-135 in which two signals appeared one at ∂ 3.32 for$O\text{-}CH_2$ (deshielded) and ∂ 2.86 for CH_3 (shielded).

The Mass spectra of the compound gave the molecular ion peak (M+.) ion peak at 510m/z. Further the peak at m/z 467 showed the elimination of isopropyl group and m/z 492 is due to removal of H_2O, and the m/z 464 favours the elimination of ethanol. The peaks at m/z 325 and 73 due to Mclafferty rearrangement favoured the position of ether and hydroxyl group at C_{12} and C_3 respectively. From the above data the chemical structure of the compound was arrived as12-ethoxy-2-methyl-hentriacontan-3-ol.

Chemical Structure of the Compound



5. Determination of Antimicrobial Activity by Agar Well Diffusion Method

The Compound was dissolved in an adequate amount of dimethylsulfoxide. Liquid nutrient agar media and the Petri plates were sterilized by autoclaving at 121 ºC for about 30 minutes at 15 lbs pressure. Under aseptic conditions in the laminar airflow chamber, about 20 ml of the agar medium was dispensed into each Petri plate to yield a uniform depth of 4mm. After solidification of the media, 24 hrs culture of Gram positive microorganisms such as *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160), Gram negative microorganisms such as *E. coli* (MTCC 1698) and *Klebsiella pneumonia* (MTCC10309*)* obtained from MTCC, Chandigarh were swabbed on the surface of the agar plates. Well was prepared by using corkborer followed with loading of 50µl and 100 µl of extracts to the distinct well with DMSO as negative control and Tetracycline as positive control. The sample loaded plates were then incubated at 37 ºC for 24 hours to observe the zone of inhibition.

Table 1: Antimicrobial activity of the Compound

S. No	Microorganisms	Zone of inhibition in Diameter (mm)						
		Compound						Standard (Tetracycline)
		15µg/mL	20µg/mL	25 µg/mL	30 µg/mL	45 µg/mL	60 µg/mL	
1.	*Bacillus cereus*	8	11	30	38	11	13	23.0
2.	*Staphylococcus aureus*	9	11	41	44	11	13	23.0
3.	*Escherichia coli*	9	12	36	42	10	12	27.0
4.	*Klebsiella pneumoniae*	8	12	41	44	10	13	22.7

From the table it was observed that with respect to the standard the maximum zone of Inhibition were found to be at 25 and 30 µg/mL concentrations against all the microorganisms tested.

6. Conclusion

On the basis of ID, 2D NMR, mass and elemental analysis data the structure of the new compound was assigned as12-ethoxy-2-methyl-hentriacontan-3-ol.

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8. References

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