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New hydroxy carboxylic acid from the root bark of *Ziziphus oenoplia*

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Abstract
Ziziphus oenoplia Linn., Mill, (Rhamnaceae) known as jackal jujube is a deciduous or evergreen thornytrees, shrubs or rarely herbs. It's root is traditionally used as astringent, bitters, anthelmintic, digestive, antiseptic and for hyperacidity, ascaris infection, stomachalgia and healing of wounds [1]. The root bark of *Ziziphus oenoplia* on extraction with chloroform solvent resulted in the identification of three new compounds. The structure of one of the compound was elucidated on the basis of spectroscopic analysis and stereochemical assignments with reference to other related compounds of known stereochemistry.

Keywords: *Ziziphus oenoplia*, Root bark, Phytochemical screening.

1. Introduction
Medicinal plants play a key role in the human health care. Infectious diseases are one of leading cause of premature death. In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world due to indiscriminate use of antibiotics [2] Even today plant materials continue to play major role in primary health care and higher plants have been shown to be potential sources for the new antimicrobial agents [3].
Ziziphus Oenoplia is a thorny sprawling bush, widely spread and has been used traditionally as antiinfectious, antidiabetic and diuretic activities [4, 5]. Phytochemical investigations have established the genus *ziziphus* (Rhamnaceae family) to be a rich source of cyclopeptide alkaloid.
To date over, 170 cyclopeptides have been published; among these 81 cyclopeptide alkaloids have been reported from various *Ziziphus* species and these include thirty five, 13-membered thirty nine14-membered and seven15-membered cyclopeptides. In continuation of the search for bioactive substances of new structural type we found that the chloroform extract from the root bark of *Ziziphus Oenoplia* consists of a new compound (Hydroxy carboxylic acid) in addition to the already reported compounds.

2. Materials and Methods
The roots of *Ziziphus Oenoplia* were collected in and around Coimbatore district and were identified by BSI, Coimbatore. The roots were thoroughly washed, dried under shade, powdered and stored in air tight container for further use.

2.1 Experimental
Melting point of extracted compound was determined by open capillaries and is 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}Hand ^{13}C NMR spectra were recorded on a Bruker DRX-400 NMR spectrophotometer using TMS as internal standard and chemical shifts were expressed in δ ppm. Mass spectra was recorded on EM-404#615 and GC-LC/MS 5970 mass spectrophotometer. Elemental analysis was performed on Vario-EL III CHNOS-Elemental analyser. Analysis results were within 0.4% of the calculated value.

2.1.1 Preparation of extracts
the pulverized dried root barks of *Ziziphus Oenoplia* was successively extracted with petether, chloroform, methanol in Soxhlet apparatus. The different extracts were tested for the presence of steroids, reducing sugars, carbohydrates, triterpenoids, alkaloids, saponins, phenolic compounds, protein glycosides, tannins & flavanoids. The results obtained for phytochemical

tests were tabulated.

Table 1: Characteristic color of root bark extract

Type of extract	Color of extract
Pet ether	Dark Brown
Chloroform	Reddish Brown
Methanol	Light Red

Table 2: Phytochemical screening on root bark of *Z.Oenoplia*

Type of Constituents	Pet ether extract	Chloroform extract	Methanol extract
COOH	+	+	+
Proteins	-	-	-
Glycosides	-	-	-
Alkaloids	+	-	+
Wax fats	-	-	-

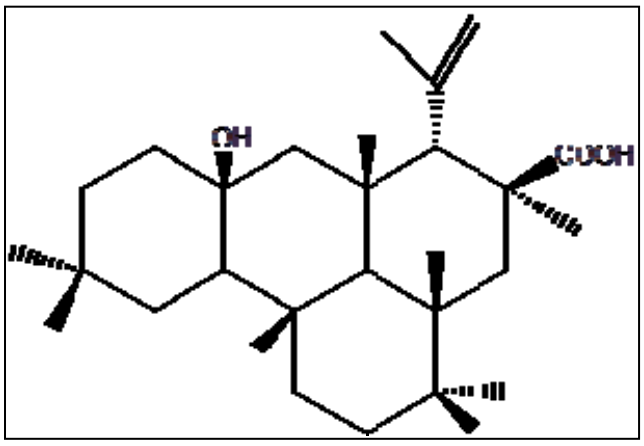
3. Results and Discussion

The $CHCl_3$ extract was selected for further chromatographic separations and resulted in the isolation of a new compound which was obtained as white amorphous powder. Its molecular formula $C_{27}H_{44}O_3$ (MW: 416) was confirmed by recording mass spectra (m/z: 416) at two different spectrophotometer, one at EM-404#615 another GC-LS/MS5970 mass spectrophotometer. The IR_{KBr} spectrum indicated the presence of hydroxyl groups, an acid carbonyl group and aliphatic rings. The ^1H NMR Spectra (DMSO-d_6) showed δ values only in the aliphatic region, which was confirmed by the test of aromaticity when kept in Nickel spatula it does not burnt with a sooty flame.

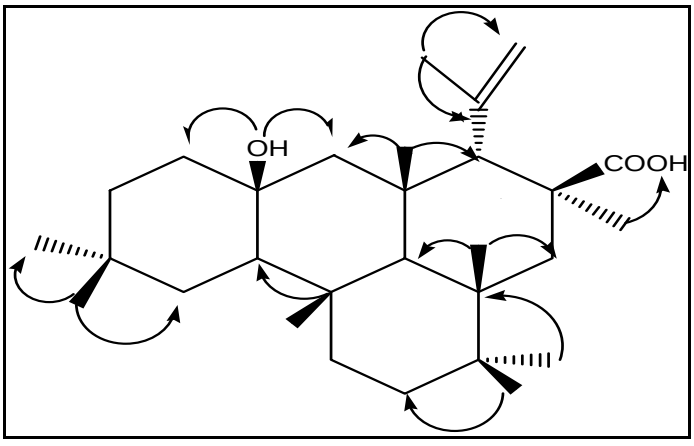
^{13}C NMR (DMSO- d_6) showed 27 carbon signals along with the presence of six quaternary carbons. Analysis of ^1H-^1HCOSY and ^1H-^{13}C HSQC,HMBC led to the assignment of the spin systems for hydroxyl and carboxylic acid groups and suggested that it was an aliphatic cyclic compound with four six membered rings. From DEPT-135, the presence of 12 odd carbons at δ 77.23, δ 55.34, δ 50.38, δ 48.99, δ 47.07, δ 38.05, δ 28.56, δ 19.40, δ16.41, δ 16.27, δ 16.19, δ 14.84 which lie above the base line; whereas eight even carbons at δ 110.11, δ 38.01, δ 36.79, δ 34.37, δ 32.16, δ 30.55, δ 29.66, δ 18.42, lie below the base line out of which the signal atδ 177.7 corresponds to COOH group respectively.

The Mass spectra of the compound followed the fragmentation pattern with M^+ ion peak at 416 m/z from which molecular formula $C_{27}H_{44}O_3$ be arrived. Further the fragmentation at m/z 387 favours the elimination of propyl moiety and m/z 371 is due to removal of –COOH. From the molecular formula and mass spectral data the molecular weight was calculated as 416, which was supported by elemental analysis, which confirmed the percentage of C, H, O to be 77.72%, 11.18%, 11.09% respectively. The presence of alkaloid was ruled out by the absence of nitrogen (Qualitative analysis) and mass spectra.

From the above data structure of the Compound 1 was identified as **7a-hydroxy-6-isopropenyl-3,3,3a,5,6a,10,10-11b,octamethyl–hexadecahydro-benzo[de]anthracene5-carboxylic acid.**



Chemical structure



HMBC Correlation

4. Conclusion

By utilizing the IR, ^1H, ^{13}C, COSY, HETCOR, HMBC, Mass, Elemental analysis and functional group analysis the compound isolated from a chloroform extract was identified as **7a-hydroxy-6-isopropenyl-3, 3, 3a, 5, 6a, 10, 10-11 boctamethylhexadecahydrobenzo[de]anthracene 5carboxylic acid** from the root bark of *Ziziphus oenopli*

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