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Isolation and identification of 1-methyl-H-Cyclopenta [b]naphthalene-4, 8-diol from the Alcoholic extract of Piper betle Linn. (Leaf stalk)

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Abstract:
Natural products have been a major source of drugs for centuries. The leaf stalk of *Piper betle* is used traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, cuts and injuries. The main objective of this work is to carry out phytochemical study of the methanol crude extract of the leaf stalk of P. betle. Phytochemical screening on this crude extract revealed the presence of phenols, alkaloids, steroids, terpenes, saponins and flavonoids. After silica gel column chromatography the crude extract led to the isolation of compounds ***PBT-I, (1-methyl-H-Cyclopenta [b]naphthalene-4, 8-diol)***. Characterization of this compound was achieved via spectroscopic methods (NMR, UV, mass spectroscopy and IR).

Keywords: *Piper Betle (*leaf stalk); IR;^{1}H NMR ; ^{13}C NMR and Mass Spectroscopy.

Introduction:

Piper betle Linn. (Local name 'Pan') belongs to family *piperaceae*, a dioecious, perennial creeper, climbing by many short adventitious rootlets, widely cultivated in hotter and damper parts of the country is wide spread in damp forests and is cultivation in India and other countries in South East Asia, such as Vietnam and China. In Ayurveda the leaf of P. betle are used as acrid, healing, tonic, carminative, stomachic, anthelmintic, aphrodisiac, laxative, bronchitis, elephantiasis of the leg and to improve appetite. But it should not be taken in eye diseases, leprosy, poisoning thirst, alcoholism and asthma. In Unani system of medicine leaves are used to improve taste, appetite, tonic to the brain, in heart and liver diseases, strengthens the teeth and clear the throat. The juice of leaves is dropped into the eye in night blindness[1]. In India, the ayurvedic system has described a large number of such medicines based on plants or plant product and the determination of their morphological and pharmacological or pharmacognostical characters can provide a better understanding of their active principles and mode of action. However a large number of tropical plants have been studied in detail for their chemical constituents, pharmacological properties of the extracts, and their pharmacognostical characterization including DNA sequencing etc.

Material and methods:

The *Piper betle* plant material was collected from Kolkata (West Bengal). The leaf stalk studied was collected from plants grown in Kolkata, West Bengal. A voucher specimen has been deposited at the herbarium of Vikram University, Ujjain (M.P.). The taxonomic identification of the plant material was obtained from the authorities of the institute of environment management and plant sciences, Vikram University, Ujjain (M.P.) India.

Extraction by Soxhlet Extractor

About 25 kg shade dried material of the plant were grinded in mechanical stirrer and squeezed to remove water. The squeezed material were dried and extracted with methanol either in cold condition or by Soxhlet extractor. The extract was dried in vacuum and subjected to TLC analysis.

Processing of *Piper betle Linn. (Leaf stalk)*

The extract was fractionated on a new technique, due to which the time and cost is reduced, in this technique the extract was coated with silica gel (60-120) mesh size in 500ml conical flask and eluted with different solvents in their increasing order of polarity. Due to these technique three fractions of different solvents namely n-hexane, benzene, and ethyl acetate are prepared. Since the yield of hexane fraction is not good and work on hexane, benzene extract was already done, so we have not taken it. The ethyl acetate fraction was taken up for the present work. The fractionated ethyl acetate was qualitatively analyzed by thin layer chromatography (TLC) to know the number of compounds present in it. The ethyl acetate elute was separated by column chromatography using silica gel (60-120) mesh size (Merck) as an adsorbent. The elution of the column was carried out with various solvents and mixture of solvents in increasing order of polarity

Result and Discussion:

Characterization of compound- PBT-I

The compound was isolated from benzene: ethyl acetate (9:1 v/v) elutes (table-1. Fr.-2).The melting point was found to be 189^0C. The mass spectrum and other spectral data revealed its molecular weight 212.24 and molecular formula $C_{14}H_{12}O_2$. It is soluble in chloroform.

IR- Spectrum (λ_{max}, KBr, cm^{-1})

The IR spectrum (KBr) showed strong broad absorption band at 3295 cm^{-1} showed the presence of hydroxyl group (-OH). The absorption bands at 2929cm^{-1} was due to aromatic –CH stretching vibrations[1], the absorption bands at 1072 cm^{-1} due secondary alcoholic group of naphthalene.

^{1}H NMR Spectrum (300 MHz, $CDCl_3$, TMS, δ)

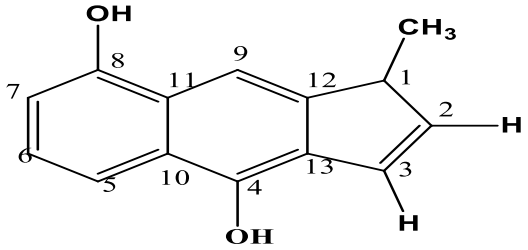
^{1}H NMR Spectrum in $CDCl_3$ showed singlet two hydroxyl protons at δ 5.0 at C-4 and C-8 position. Peak at δ1.604 singlet revealed three protons of methyl group attached to C-2 of Cyclopentyl ring. At δ3.7 ppm showed doublet generated proton at C-1 of Cyclopentyl ring. Absorption at δ6.074 and δ6.548 showed triplet and doublet proton at C-2 & C-3 of Cyclopentyl. The rest of the four protons of aromatic ring shows doublet at δ7.025 C-7, triplet at δ7.344 C-6, δ7.915 doublet resonating at C-5 and δ7.902 a singlet signal at C-9.

^{13}C NMR Spectrum (500 MHz, $CDCl_3$, TMS, δ)

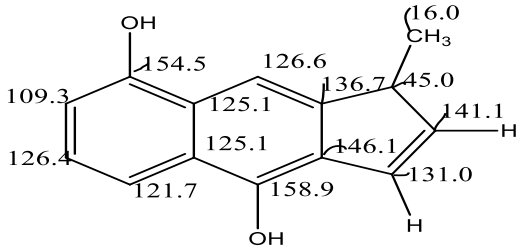
^{13}C NMR Spectrum recorded in the $CDCl_3$ in 500 MHz. The peak at δ16.0 is methyl carbon attached to Cyclopentyl ring at C-1 and δ45.0 (C-1) showed peak of Cyclopentyl-CH group. The peaks at 109.3(C-7), δ 121.7(C-5), 125.1(C-10 and C-11), 126.4(C-6), 126.6 (C-9)1, 36.7(C-12)., 131.0(C-3), 141.1(C-2) , 146 (C-13) are due to aryl carbons. And peaks at δ154.3 (C-8) and δ158.2 (C-4) are due to the carbon atoms attached with –OH group in the naphthalene moiety.

Mass Spectrum (EIMS, m/z, rel. int. %)

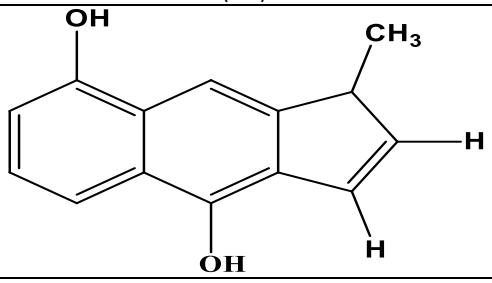
The mass spectrum showed molecular ion peak at m/z 212 corresponds to molecular formula $C_{14}H_{12}O_{12}$. The base peak at 179 m/z 100% abundance. Other mass fragments are 54.05, 158.4 shown by scheme-I. Thus on the basis of above evidences the compound PBT-I is identified as **1-methyl-H-Cyclopenta [b]naphthalene-4,8-diol.**



1-methyl-H-Cyclopenta [b]naphthalene-4,8-diol



^{13}C NMR spectral data of 1-methyl-H-Cyclopenta [b]naphthalene-4,8-diol

Molecular formula	$C_{14}H_{12}O_{12}$
Melting point	189^0C
Molecular ion peak	212 m/z
TLC solvent system	Hexane: Benzene (0.5:9.5, v/v)
Recrystallization	Methanol
Solubility	Chloroform
State	Dark Brownish amorphous solid
IR- Spectrum (λ_{max}, KBr, cm^{-1})	3295(-OH of Phenol) 2929.08 (CH-str.), 2353, 2049,1606.38 (C=C str.), 1393, 1072 (2^0 -OH) cm^{-1},
^{1}H NMR Spectrum (300 MHz, $CDCl_3$, TMS, δ)	δ 1.604 (s,3H,CH_3), 3.7 (d,1H, Cyclopentyl-CH), 5.0 (s, 2H, 2x OH), 6.074(t,-CH), 6.548(d,-CH), 7.915 (d, 1H, Ar-CH), 7.344(t, -CH), 7.025(d,-CH), 7.902(s, -CH).
^{13}C NMR Spectrum (500 MHz, $CDCl_3$, TMS, δ)	45.0,141.1, 131.0, 158.9, 121.7, 126.4, 109.3, 154.5, 126.6, 125.1, 136.7, 146.1, 16.0 ppm
Mass Spectrum (EIMS, m/z, rel. int. %)	M+ 212.08 (55), 195 (3.2), 179 (99), 133 (7.0), 101 (9.21), 89 (1.1)213.09, 63 (1.0).
Structure of the compound	
IUPAC Name	*1-methyl-H-Cyclopenta [b]naphthalene-4,8-diol*

Isolation of compound 1-methyl-H-Cyclopenta [b]naphthalene-4,8-diol (PBT-I)

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