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Original Research Article

ISOLATION AND IDENTIFICATION OF ALIPHATIC COMPOUNDS FROM THE BENZENE EXTRACT OF *PIPER BETLE* Linn. (LEAF STALK)

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Abstract: The benzene extract was separated by column chromatography using alumina grade (iii) as adsorbent. Alumina was deactivated o with 7% water before filling in the column. The elution of the column was carried out with the various solvents and mixtures of solvent increasing order of polarity. The rechromatography of various fractions afforded aliphatic compounds in pure form designating as BNA-3 [pentatriacont-6-ol(7)} and BNA- 4 [tetratriacontanol(8)]).The compounds were identified by using IR, $^{1}H_{NMR}$, $^{13}C_{NMR}$ and mass spectroscopy and the various present in isolated compounds are tested by Feigl test , alkaline hydrolysis, cerric ammonium nitrate test and tetranitro methane test(TNM).
Key words: piper betle (leaf stalk), IR, 1 H_{NMR}, $^{13}C_{NMR}$ and mass spectroscopy.

Introduction: The family of *piperaceae* belonging to super order *Nymphaei flloraea* order *piperales* and genus *piper* of family *piperaceae* commonly known as pan comprises about 10 genera ,2000 species.T genus piper is largely distributed in tropical and subtropical regions of the world[1].Over 700 species of *piper* betle has been distributed in both of the hemispheres of world. Of these, 30 species have been recorded in India, 18 in srilanka and 3 are endemic. Piper betle is cultivated in India, srilanka, Malaysia, Indonesia, Philippine islands and east africa[2].

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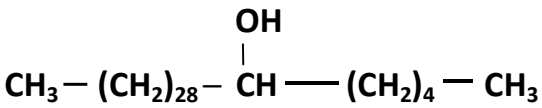
The parts of piper betle utilized, are leaves, roots, stems, stalks and fruits. Piper betle has light yellow aromatic essential oil, with sharp burning taste. Leaf possess activity like antidiabetic, antiulcer, antiplatelet aggregation,antifertility,cardiotonic,antitumour,antimutagenic,respiratory depressant and antihelminthic[3-11].piper betle is used to treat alcoholism ,bronchitis, asthma, leprosy and dyspepsia. Earlier, anti-ulcerogenic activity of piper betle was attributed to its antioxidative property.a prelimary study has reported piper betle leaves extracts contains large number of bioactive molecules like polyphenols, alkaloids, steroids, saponins and tannins[12]. The leaves extract of piper betle have also been reported to exhibit biological capabilities of detoxication,

antioxidation and antimutation that suggested the chemopreventive potential of extracts against various ailments including liver fibrosis and carcinoma[13].

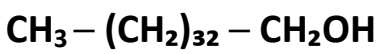
Materials and Methods: Plant Material: The piper betle plant material was collected from Kolkata (West Bengal). The leaf stalk studied was collected from plants grown in Kolkata, West Bengal. A voucher specimen has been deposited at the herbarium of Vikram University Ujjain (M.P).The taxonomic identification of the plant material was obtained from the authorities of the institute of Environment Management of plant sciences, Vikram University Ujjain (M.P).India.

Experimental: Extraction and Isolation: The benzene extract as prepared was separated by column using alumina grade(iii) as adsorbent . Alumina was deactivated with 7% water before filling in the column. The elution of the column was carried out with various solvents in increasing order of polarity. The rechromatography of various fractions yielded two compounds in pure form , which are designated as **BNA-3 [pentatriacont-6-ol(7)] and BNA-4 [tetratriacontanol(8)].**The various groups present isolated compounds are tested by Feigl test ,Alkaline hydrolysis , Cerric ammonium nitrate test and tetranitromethane test (TNM).

Identification of compounds: The identification of the compounds present in benzene extract was analyzed by TLC, which revealed the presence of various spots. To separate it the extract was subjected to column chromatography using alumina grade (iii) as adsorbent .The column was eluted with different solvents in their increasing order of polarity. The column afforded two compounds in pure form designated as PBT-3 and PBT-4



BNA-3



BNA-4

Compound BNA-3:

Colourless crystals M^+508,M.F. $C_{35}H_{72}O$, M.P. 85°C,Yield 90mg. IR spectrum (KBr) showed a strong absorption band at 3425 cm^{-1} was due to the presence of hydroxyl group. Bands at 2925, 2850 and 1475 cm^{-1} were due to C─H stretching and bending vibrations. Bands at 1060 -1050 and 730-720 cm^{-1} showed aliphatic long chain nature of alcohol.

^1H NMR spectrum in $CDCl_3$ showed that the terminal methyl group protons were resonated at δ 0.80 (J=6.0 Hz) as triplet. The –OH and four methylene protons β – to –OH group were resonated at δ 1.55 as broad singlet.

Methane proton resonated at δ 3.60 (J=8.0Hz) as multiplet and a broad singlet at δ 1.20 integrating for 60 protons for 30 methylene groups.

Mass spectrum showed it to be along chain aliphatic alcohol . It showed the molecular ion peak at m/z 508. The abundant peaks at m/z 437 and m/z 407 were due to α- cleavage indicating the position of –OH group at C-6. An intense peak was observed at m/z 451 & m/z 393 due to β-cleavage with respect to –OH group .Other peaks appeared at an interval of 14 mass units i.e. by regular loss of - CH□ groups.

Thus on the basis of above spectral data compound **BNA-3** was identified and

characterized as **Pentatriacont-6-ol**[14]. It is new compound and being reported first time by author.

Compound BNA-4:

M^+ 494, M.F.$C_{34}H_{70}O$, M.P. 140^{O}C and yield 130 mg, shows negative test with TNM and red colour cerric ammonium nitrate indicating the saturated nature of the alcohol .

IR spectrum in (KBr) showed stretching and bending vibration at 3440 cm^{-1} and 1060^{-1} respectively indicating the presence of of-OH group .Peaks 2920, 2860, 1480 and 1460cm^{-1} were due to C-H stretching and bending vibrations. The absorptions at 730 and 720 cm^{-1}, characteristic of $(CH_2)_n$ skeleton vibration i.e . The compound was a long chain aliphatic alcohol.

^{1}H NMR spectrum in $CDCl_3$ showed a triplet at δ 0.82 (J=7.0Hz) for three protons of the end methyl group. A triplet at δ 3.58 (J=8.0 Hz) for two protons was due to methylene attached to the –OH group. The –OH proton resonated at δ 1.50 as a singlet. The rest of the 32 methylene group protons were resonated at δ 1.19 as broad singlet.

Mass spectrum showed the molecular ion peak at m/z 494. It's molecular

Formula was found to be $C_{34}H_{70}O$. The fragment ion at m/z 447 was base peak due to loss of M- 47 was base peak due to loss of M-47(M-H_2O+CH_2=CH_2=H) indicated the presence of primary alcohol .An intense peak observed at m/z 451 was due to loss of (M^+-43).

13C NMR Spectrum shows a sharp peak at 63.07 ppm corresponds to the carbon attached to the -OH group (-CH_2OH). The peak at 32.83 and 31.89 ppm correspond to the methylene carbons attached at the α and β position of the primary alcoholic group. The peak at 13.99 ppm indicated the terminal methyl group. The peak at 22.63 and 25.73 ppm were assigned to the α and β carbons of the methylene groups attached to the methyl group. A bunch of peaks at 29.65 ppm correspond to the remaining methylene cabons

Thus on the basis of above spectral data compound **BNA-4** was identified and characterized as **tetratriacontanol**[14].

Results and Discussion:

The leaf stalks were grinded in mechanical stirrer and squeezed to remove water the extract was dried in vacuum and subjected **TLC** analysis. TLC showed the presence of two compounds by showing various spots in different solvent systems in increasing order of polarity. The rechromatography of various fractions afforded two aliphatic compounds in pure form designated as PBT-3 &PBT-4.

M^+508, $C_{35}H_{72}O$ (Hexane: benzene eluate (9:1,V/V),M.P.85^{0}C,

Yield-90mg,colourless crystals, Column rechromatography adsorbent-Alumia grade iii, TLC solvent system Hexane:ether:ethanoic acid (8.5:1.5: .5 v/v), TLC spot single, solubility in chloroform, showing negative TNM test and positive cerric ammonium nitrate.

IR Vmax

[KBr]:3425, 2925, 2825, 1475, 1025, 1060-1050, 730-720 cm^{-1}.

^{1}H NMR [300 MHz, $CDCl_3$,TMS, δ]: 0.80 (t, 6H, 2X CH_3) 1.20 (brs, 60H, 30xCH_2) 1.55 (s, 1H ,-OH and 4H-2βxCH_2) 3.60 (m, 1H

EIMS (m/z , rel. int.) 507(1), 479(14),451 (23), 437 (8), 423(12), 394(0.5), 380(4), 328 (4),299 (1), 277(8), 253 (12), 241 (4),209 (4) ,185(9), 171 (5),129 (20), 125 (12), 111(19), 105(41),97(44), 96(20), 85 (36),83 (44), 82 (30), 77(21), 71(68) , 69(34),60(18),57(100),55(59).

M^+494, $C_{34}H_{70}O$, (Hexane:benzene eluate (8.5: 1.5,V/V) M.P.140^{0}C,

Yield-130mg, state colourless crystals, solubility-chloroform,

TLC solvent system Hexane:ether:ethanoic acid (8.5:1.5:5,V/V)

Test; Negative TNM & Positive cerric ammonium nitrate

IR V_{max} [KBr]: 3440, 2920, 2860, 1480, 1460, 1060, 730-720 cm^{-1}. **^{1}H NMR [300 MHz, $CDCl_3$,TMS,δ]:** 0.82(t, 3H, -CH_3) 1.19 (brs, 64H,

32xCH_2), 1.50 (brs, 1H, -OH) , 3.58(t , 2H ,-OCH_2).

EIMS (m/z, rel. int.) M494(.25), 493(0.25), 479(4), 451(8), 447(10) ,419(8) , 391(4), 362(1) , 334(2), 299(2), 253(5), 237(3), 223(4), 209(5), 195(6), 181(8), 167(8), 153(10), 139(12), 125(20), 111(14), 105(8), 97(59), 96(21), 85(39) , 83(71),82(41), 81(17), 71(64), 69(60), 68(27), 67(14), 57(100), 55(80).

^{13}C NMR [75 MHz, $CDCl_3$,TMS, ppm] : 13.99, 22.63, 25.73, 29.65, 31.89, 32.83, 63.07 pmm.

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