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# STEROIDS FROM STEM (BARK) OF *BUTEA MONOSPERMA*

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## ABSTRACT

The chemical examination of benzene extract of the stem bark of B. *monosperma* revealed the presence of steroids for isolation of steroids the stem bark of *B. monosperma*. dried powder of stem bark of *B. monosperma* was subjected to hot extraction with benzene. The benzene extract was subjected to column chromatography. Isolated compounds were purified and crystallized by chloroform: methnol. After isolation and purification afforded white crystalline substance which was subjected to physical , chemical and spectral analysis and identified by IR, $^{1}$H-NMR, $^{13}$C-NMR and higher resoluation mass spectra. The compound identified as β-sitosterol(1) and stigma sterol(2).

**KEYWORDS:** *Butea monosperma* ; stem bark; β –sitosterol , stigmasterol.

## INTRODUCTION

Butea monosperma (Lam.)/Kuntze (bark), locally known as palas belongs to family leguminoceae. The plant has been popularly use for curing various diseases. The plant has been reputedly used as tumour, skin diseases, piles, urinary discharge, wound healing. Used in traditional medicine[1-4]. Its seed and flowers have been found to posses anthelmintic and antifertility,antifungal activity [5-6] .Its seed, flower, leaves ,bark and whole plant are used as medicine. Its chemical constituent terpenes, wax, gum flavonoids, glycoside, cyaniding, alkaloids [7-11]. In this paper we have report the isolation and characterisation of known compound. first time isolated from benzene extract of *B. monosperma* (bark), namely β –sitosterol and stigma.

## RESULT AND DISCUSSION

From the positive tests for steroids given by compound (1) and (2) at is determined to be a compound containing steroidal nucleus, The compounds are white crystalline needle shape

MP 138-139$^{o}$C, 140-142$^{o}$C. Compound (1) IR spectroscopic analysis, the observed absorption bands are (3425cm$^{-1}$) that is characteristic of (O-H stretching). Absorption at 2937 cm$^{-1}$ and 2869cm$^{-1}$ is due aliphatic C-H stretching other absorption frequencies include 1621cm$^{-1}$ as a result as C=C stretching weak band at 1463cm$^{-1}$ is a bending frequency for cyclic $(CH_2)$n and 1379cm$^{-1}$ for $–CH_2$ $(CH_3)_2$.The out of plane C-H vibration of unsaturated part was observed at 958, 735-10cm$^{-1}$ [13,14,15]. The resemble the absorption frequencies observed for, β- sitosterol.

$^{1}$H-NMR showed the singnal at ð0.67(s) and ð1. 008(d) for two angular methyl groups. A doublet at ð 0.94 was assigned to C-21 methyl protons. The carbinolic proton was assingnrd at ð 3.5(m) . Abroad doublet at ð 5.35(dd) was assigned to olefinic proton at C-6. A singlet at ð1.51(S,H) confirmed the presence of- OH Group. While the multiplet at ð3.5(m) was assigned the presence of C-3-proton. A triplet at ð0.84 was assingned the methyl protons of C-29 [16, 17]. The $^{13}$C NMR has shown the signals at 139.0 & 121.4ppm which are assingned C-5 and C-6 double bonds respectively as in D5 steroids. The terminal and angular methyl carbon C-18, C-19, C-21, C-26 and C-29 were assingned at 11.7, 18.75, 18.60, 19.53 and 11.9ppm corresponding. The carbinolic carbon at C-3 position assigned at 71.5ppm [15, 20, 21].

Mass spectrum showed molecular ion peak at m/z 414.The mass spectrum showed characteristic patter of steroidal molecule bands at m/z 396( $M+H_20$), 273(M+Side chain ) and other fragments were inconsistent with the proposed structure [18, 19].

Compound (2) The IR absorption spectrum showed absorption peak at 3428cm$^{-1}$( O-H stretching), 2940cm$^{-1}$ and 2867cm$^{-1}$ (aliphatic C-H stretching).1639cm$^{-1}$(C=C) absorption peak other absorption peaks includes 1461cm$^{-1}$ $(CH_2)$, 1380cm$^{-1}$(OH)10 54cm$^{-1}$(cycloalkane) and 958cm$^{-1}$ These absorption frequencies observed for steroidal molecule. $^{1}$H-NMR showed the signal at ð0.67(s) and ð 0.92(d) for two angular methyl groups. A doublet at ð 0.84 was assigned to C-21 methyl protons. The carbinolic proton was assingnrd at t ð3.5(m). Abroad doublet at ð5.35(dd) was assigned to olefinic proton at C-6. A singlet at ð 1.51(S,H) confirmed the presence of- OH group . While the multiplet at ð3.52(m) was assigned the presence of C-3proton. A pair of double doublet at C5.15 (J=6&7HZ) and ð 5.02 (J=6&7) [15, 16] was assigned to the double bond present at C-22 in side chain.assigned the methyl protons of C-29. the presence of C-3-proton. A triplet at ð0.84 was assingned the methyl protons of C-29.

The $^{13}C$ NMR has shown the signals at 138.0 & 121.4ppm which are assingned C-5 and C-6 double bonds respectively as in $\Delta^5$ steroids. The signal at 138.0and 129.0 ppm resonated double bond in side chain at C-22and C-23position. terminal and angular methyl carbon C-18, C-19 , C-21 , C-26 C -27and C-29 were assingned at 11.7, 18.74, 18.60, 22.7, 19.5 and 11.9ppm corresponding. The carbinolic carbon at C-3 position assigned at 71.5ppm. [15,20-22]

Spectrum: Mass spectrum showed molecular ion peak at m/z 412.suggesting molecular formula as $C_{29}H_{48}O$ The mass fragments at m/z 394($M+H_20$), 273(M+-Side chain) 255(M+-side chain +$H_2O$) and other fragments were inconsistent with the proposed structure. [18, 19].

**Experimental**: collection, identification and preparation of plant materials (extract) The bark of plant were collected from the nearby area of Ujjain city in month of March. The plant material bark was identified from school of studies in Botany Vikram University Ujjain. The bark of Plant was shaded, dried and powdered.

**Extraction and Isolation**: powdered (15kg) bark of B. monosperma was extracted exhaustively with benzene (79$^oC$) in a soxhlet extractor. The solvent was recovered by rotator evaporator, under vaccum pressure, to efford dark greenish brown oily mass (165gm) which was labeled benzene extracts of B .monosperma and kept in the refrigerator.

**Chromatography Separation**

The benzene extract was subjected to TLC examination using (benzene: ether: acetic acid (8:2:0.5, v/v). The benzene extract showed positive test for steroidal nucleus. This extract was fractionated on alumina gel. III column. The column was run using hexane, hexane: benzene, benzene with increased polarity. The hexane: benzene fractions and benzene fraction were collected in Bulk and monitored by TLC examination. And showed single spot which was further purified and crystallized using (Chloroform: Methenol) yielded white crystalline needle shape crystal with melting point 138-139 $^0C$ and 140-142$^0C$ respectively, Was further subjected to IR , $^1H$ NMR (300$MH_Z$) ,$^{13}C$-NMR (75$MH_Z$) and mass spectra to ascertain the chemical structure.

**Tests for Steroid**

**Salkowski Reaction**: a few crystals were dissolved in chloroform and few drop of conc. $H_2SO_4$ were added to the solution. A reddish color developed in $CHCl_3$ layer [12].

**Liebermann-Burchard Reaction**: a few crystals were dissolved in chloroform and few drop of Conc. $H_2SO_4$ were added to it followed by Addition of 2-3 drops of acetic anhydride solution turned violet blue and finally green [12].

**Spectroscopic Characterization:** Different spectroscopic method were used to elucidate the structure of isolated compound (1) and (2) among the spectroscopic techniques IR, $^1$H-NMR, $^{13}$C- NMR ,mass Spectra were carried out IR spectra was recorded in KBr on perkin Elmer-377. The $^1$H-NMR spectra was recorded on 300$MH_Z$ XL spectrometer and 400MHz Brucker WM spectrometer with TMS as internal standard,$^{13}$C –NMR spectra was recorded on varian XL75 $MH_Z$ spectrometer in $CDCL_3$.The mass spectra was recorded on Jeol–JMSD-300 mass spectrometer. The column chromatographies were carried out on alumina Gr.!!! And TLC on silica gel. The spot was visualized by exposure to iodine vapour or charring with conc. $H_2SO_4$ - Vanillin spray.

Compound (1) The IR spectrum ($\lambda_{max}$.,KBr, $cm^{-1}$) the IR spectrum showed absorption peak at 3425, 2938, 2869, 1621, 1463, 1379, 1054, 958, & 735-10 $cm^{-1}$.

$^1$HNMR($CDCL_3$ 300$MH_Z$, ð)$^1$HNMR has given signals at ð0.67(3H,S,CH3), ð1.008(3H,s,CH3) ,ð0.94(3H,d,$CH_3$-C21), ð0.84(m,9H), ð1.51(1H,S,OH),ð3.5(1H,m,H-3), ð5.35(H,bd.H-6)

$^{13}$CNMR ($CDCl_3$, 75$MH_Z$) $^{13}$-CNMR has given signal at 139.4(C-5), 121.4(C-6), 71.5(C-3) 56.47(C-14),55,76(C-17), 49.8( C-9), 45.8(C-1),45.5(C-13), 42.0(C-24), 39.48(C-12),36.9(C-1),36.21(C-4),35.86(C-10),33.65,33.4(C-20,C-22),31.36,(C-7),31.6(C-8),28.86(C-25), 27.96 (C-2),25.12,25.78(C-23,C-16)),24.01(C-15),22.7(C-28),20.79(C-11),19.11,19.53(C-26,27), 18.75.18.60(C-19,C-21),11.7,11.9(C-18,C-29).

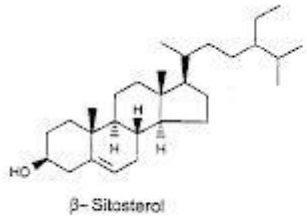
EIMS: m/z( rel.int.,%): Mass spectrum showed molecular ion peak at m/z 414 and molecular formula $C_{29}H_{50}O$ other ion peaks were also observed at $M^+$ 414(28.13), 400(59.0), 396(42.0), 382(24.5), 367(20.0), 329(38.0), 315(22.0), 303(40.0), 273(34.6), 255(46.0), 231(32.4),213(35.7), 122(26.8), 118(25.0), 107(22), 105(42), 95(42.0), 84(36.0), 54(74), 43(100).

Compound (2) The IR spectrum ($\lambda_{max}$.,KBr, $cm^{-1}$) the IR spectrum showed absorption peak at 3428, 3425,2940, 2868, 16239, 1461, 1380,1243,1190,1056, 834, & 735-10 $cm^{-1}$ .

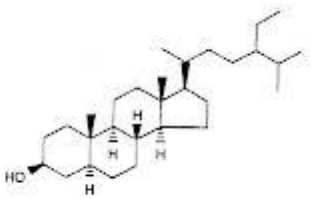
$^{1}$HNMR($CDCl_3$,300$MH_Z$ ð)$^{1}$HNMR has given signals atð0.67(3H,S,$CH_3$), ð1.008(3H,s,$CH_3$), ð0.92(3H,d,$CH_3$-C29), ð0.84(m,9H), ð1.51(1H,S,OH),ð3.5(1H,m,H-3) ð5.35(H,dd.H-6)

$^{13}$CNMR($CDCL_3$,75$MH_Z$) $^{13}$CNMR has given signal at138.0(C-5,C-23),129(C-22),121.4( C-6), 71.8(C-3),5 6.47(C-14), 55,76(C-17), 49.8(c-9), 45.54(c-24), 42.0(C-13), 39.48(C-12),(C-4), (C-20), 36.2,36.9(C-10,C-1),31.6(C-7),31.36(C-8),28.86(C-25),27.96(C-2),24.01(C-15), 22.7 (C-26), 25.76(C-16), 20.79(C-11), 19.5(C-27), 18.74, 18.60(C-19,21) 11.5,11.7,11.9(C-28,C-18, C-29).

EIMS: m/z( rel.int.,%): Mass spectrum showed molecular ion peak at m/z 412 and molecular formula $C_{29}H_{48}O$ other ion peaks were also observed at $M^+$ 412(84.0), 400(37.0), 396(23.5), 382(25.5), 367(15.4), 329(20.0), 315(19.0), 303(22.7), 273(24.6), 255(43.0), 231(26.0), 213(26.0), 199(17.8), 173(20.2), 159(42.7), 145(54.0), 133(39.7), 129(13.7), 107(22),



β–Sitosterol



Sitostanol

## CONCLUSION

On the basis of above evidences that both compound isolated from benzene extract of B. monosperma(bark)and their structure were identified on the basis of physico-chemical analysis methods.

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