EXTRACTION AND CHARACTERIZATION OF FLAVONOIDS FROM *BUTEA MONOSPERMA* (LAM.) KUNTZE FLOWERS

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ABSTRACT

Flavonoids were extracted from the dried flowers of *Butea monosperma* (Lam.) Kuntze as: Butein, monospermoside, isobutrin and butrin. The structure of flavonoids compound have been elucidated by UV, IR, NMR and Mass spectroscopy. Flower flavonoids are uses in the traditional system of medicine for the treatment of Hepatitis-B virus and liver disorder.

Keywords: Flower flavonoids, Butein, Monospermoside, Isobutrin, Butrin from *Butea monosperma*

INTRODUCTION

Butea monosperma (Lam.) Kuntze plant[1,2] belongs to the family Papilionaceae or Fabaceae and commonly called as *Palash, Dhak, Tesu, Flame of the forest*. Plant is a moderate size upto 5-20m in height and widely distributed in Tropical Asia upto an altitude of 4000 ft. It occurs in India, Indonesia, Nepal, Thailand, Japan, Myanmar, China and Sri Lanka. It grows on open grass lands and scattered in the mixed forest. Plant are used for the production of timber, resin, fodder, medicine and dye[3,4]. Flowers of *Palash* have been reported to contain many flavonoids[5,6] and also have wide medicinal values as a folk medicine. The flowers are used to cure for liver disorders, gall bladder and skin disease(7). Flowers are also used in the traditional system of medicine for the treatment of liver disorder and Hepatitis-B virus. The antihepatotoxic principles of flowers by using assay model of carbon tetra chloride (CCl_4) and D-galactosamine including liver lesion in vitra and they claimed that Butrin and Isobutrin were responsible for antihepatotoxic properties[7]. A mixture of *Butea monosperma* (Lam) and *Tacca aspera* flowers in equal amount is especially used by the traditional medical practitioners for the treatment of Hepatitis-B virus and liver disorder.

MATERIALS AND METHODS

The melting points are uncorrected at ^1H 500 & 400MHz and ^{13}C 125 & 100 MHz, NMR Bruker DPK-400 and DMSO with TMS and in CD_3 OD, CD_2 HOD peak at 3.34 ppm, ESI-MS, TSQ 700 MAT Mass Spectrometer, UV-240 in Methyl alcohol. The shift reagents were prepared by standard procedure, FT-IR, Perkin Elmer model G Y system in KBr. Column Chromatography were prepared by using silica gel and Thin Layer Chromatography 0.25mm precoated silica gel and spots were detected by inspection under UV light (254nm) by spraying Ferric chloride solution (5%) and exposed to ammonia vapour without ultraviolet light.

Flowers used in this study were collected from Mathura and Agra District in the month of January to March. The plant was identified as *Butea monosperma* (Lam.) Kantze *(Palash)* at Department of Botany, SLS, Khandari Campus, Dr. Bhimrao Ambedkar University, Agra and Department of Botany, B.S.A. College, Mathura, U.P. (INDIA).

Air dried flowers powder (50gm) of *Butea monosperma* (Lam.) was isolated and extracted with ethanol (30ml) in a Soxhlet Extraction Apparatus for 24hrs. After removal of the solvent and extracted with petroleum ether ($60\text{-}80^0C$) to remove the waxy materials and obtained residue (9.70gm). The dewaed residue

was then dissolved in distilled water (50ml) and extracted with diethyl ether (150ml), ethyl acetate (150ml) and butyl alcohol (150ml). The individual solvent fractions of diethyl ether, ethyl acetate and *n*-butyl alcohol were distilled under reduced pressure to provide dried extract: 0.5612gm, 0.9254gm and 5.5126gm respectively.

The crude ether extract was dissolved in ethanol (20ml) and absorbed on silica gel (5gm), dried and silica gel was placed on the top of silica gel column to obtain a uniform layer. Column was eluted with dichloromethan (100ml) and solvent was gradiently changed from dichloroethan-diethyl ether in 4:1 or 1:1 molar ratio. Chromatography was monitored by silica gel, TLC using dichloroethan-diethyl ether in 2:1 molar ratio as solvent system. Fractions gave similar TLC pattern were combined together and concentrated, obtained 4 major fractions. Butein (1) had *Rf*=0.42 was isolated by recrystallisation with ethanol.

Ethyl acetate crude was dissolved in ethanol (20ml) and absorbed on silica gel (5gm). It was placed on the top of silica gel column with silica gel (30gm) in diethyl ether. Column was eluted with diethyl ether and ethyl acetate then ethanol. Column chromatography was monitored by silica gel, TLC using ethyl acetate-ethanol in 9:1 molar ratio as solvent. Fractions gave same spot on chromatogram, *Rf* = 0.56 were combined and recrystallized with ethanol to provide monospermoside (2).

Crude extract of *n*-butanol was dissolved in hot methanol, after cooling at room temperature, a small amount of diethyl ether was added into the solution and kept for 3 days in refrigerator. The white solids was obtained after recrystallisation with methanol to provide colorless needless shape Butrin (4). The main authentic crude solution was ether identified on silica gel column chromatographic analysis using increasing amount of ethanol in ethyl acetate. The obtained fraction had *Rf* = 0.2 were combined together after recrystallisation with ethanol to provide Isobutrin (3).

RESULTS AND DISCUSSION

Flavonoids from *Butea monosperma* (Lam.) Kuntze *(Palash*) flowers were extracted and characterized as: Butein (2^1, 3, 4, 4^1-tetrahydroxy chalcone); monospermoside (2^1, 3, 4, 4^1-tetrahydroxy chalcone-3-monoglucoside); Isobutrin (2^1, 3, 4, 4^1-tetrahydroxy chalcone-3, 4^1- diglucoside) and Butrin (4^1, 5^1, 7-trihydroxy flavanone-5^1, 7 diglucoside). These flavonoids were identified as follows :

1. **Butein:** It is orange yellow needles yield (17mg, 0.0342%), m.p. 212^0C (ethanol), λ max 260 and 380, (+NaOH) 276, 442, (+Al Cl_3) 320, 482, (+$AlCl_3$) 320, 482, (+$AlCl_3$) 270, 426, (+NaOHAc) 263, 384nm, λ max 3296 (OH), 1636 (CO), 1590, 1596, 1512 cm-^{1} (C=C) δH 6.30 (^{2}H, d, 3^1-H), 6.45 (^{1}H, dd, 5^1-H), 6.8 (1H, d, 5-H), 7.1 H (1H, dd, 6-H), 7.2 (1H, d, 2-H), 7.56 (1H, d, α-H), 7.76 (1H, d, β-H) and 8.02 (1H, d, 6^1H), δC 105, 110, 116, 117, 117.6, 120, 124.6, 130.4, 134.2, 147.8, 148, 151.2, 167.6, 168.6 and 194.6, ES 1- MS m/z (%) 272 [M+H] (100), 163 (62), 130 (85).

2. **Monospermoside:** It is a yellow needles, yield 21mg, 0.042%, m.p. 183-188^0C (ethanol), λ max 260 and 370, (+NaOH) 279, 437, (+$AlCl_3$)262, 430, (+$AlCl_3$)264, 428, (+NaOHAc) 254, 376, (NaO Ac/H_3BO_3) 254, 376nm, γ max 3350 (OH), 1634, 1630 (CO), 1508 cm^{-1} (C=C), δH 6.30 (1H, d, 3^1 -H), 6.44 (1H, d, 5^1 -H), 6.8 (1H, d, 5-H), 7.14 (1H, dd, 6-H), 7.2 (1H, d, 2-H), 7.56 (1H, d, -H), 7.76 (1H, d, β-H) and 8.02 (1H, d, 6^1H), □C 64, 72.6, 76.1, 78.8, 79.8, 105, 105.2, 110.4, 115.8, 118.6, 119.2, 120.2, 128.4, 129.7, 134.8, 146.6, 148.4, 152.6, 167.8 168.8 and 194.6, ESI-MSm/r (%) 434 $[M^+H]^+$ (100), 456 $[M+Na)^+$ (96), 273 (84), 292 (84) and 890 (20)
3. **Isobutrin:** It is a yellow needles shape compound, yield 20mg, 0.042%, m.p. 190^0C (ethanol), λ max 254 and 376, (+NaOH) 452, (+$AlCl_3$) 428, (+$AlCl_3$) 430, (+NaOAc) 382, (NaOAc/H_3BO_3) 380nm, γ max 3416 (OH), 1628 (CO), 15200m$^-$) (C=C), δH 6.56 (1H, d, 3-H), 6.60 (1H, dd, 5^1-H), 6.86 (1H, d, 5-H), 7.42 (1H, m, 6-H), 7.70 (3H, m, d -, β- and 2-H) 8.22 (1H, d, 6^1-H) and 13.52 (1H, SBR, phenolic OH), δC 64, 72.6, 76.2, 78.8, 79.8, 105, 105.4, 110.4, 115.8, 118.6, 119.4, 120.4, 128.6, 129.6, 134.8, 146.6, 148.7, 152.6, 168, 168.6 and 194.6.
4. **Butrin:** It is a colourless needles, yield 70mg, 0.14%, m.p. 196-197^0C (MeOH) and

m.p. 190-192^{0}C (MeOH)[73] λ max 274 and 312 (+NaOH) 438, (+$AlCl_3$), no shift (+$AlCl_3$) no shift (+NaOAc), no shift (NaOAc/H_3BO_3), no shift, γ max 3420 (OH), 1650 (CO), 1610 cm^{-1} (C=C).

REFERENCES

1. Sathe, P.G. and Magarkar, K.M., *Mycopathologia and Mycophologia Applicata, 51,* 159-162 (1973).
2. Shukla, R.; Chakravarty, M. and Gautam, M.P., *Journal of Medicinal Plant Research, 2(12),* 356-360 (2008).
3. Kirtikar, K.R. and Basu, B.P., *Indian Medicinal Plants,* International Book Pub. Dehradun, *2,* 1336-1339 (1987).
4. Satyavati, G.V.; Gupta, A.K. and Tandon, N., *Medicinal Plants of India,* ICMR, New Delhi, India, (1987).
5. Puri, P. and Seshadri, T.R., *J. Science Industr. Res., 12-B,* 462-466 (1953).
6. Gupta, S.K.; Ravindranath, B. and Seshadri, T.R., *Phytochemistry, 9,* 2231-2235 (1970).
7. Wagner, H.; Geyer, B.; Fiebig, M.; Kiso, M. and Hikino, H., *Planta Med., 52,* 77-79 (1986).