Investigation of Bioactive Constituents and the Antitumour principle from *Butea monosperma*

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Abstract- The ethanol extract of the flower of *Butea monosperma* (Fabaceae) was found to contain butein (1), butin (2), monospermoside (3), medicarpin-3-O-glucoside (4), ß-sitosterol glucoside (5), sulphurein (6), isomonospermoside (7), isocoreopsin (8), butrin (9), isobutrin (10) ergost-5-en-3ß-ol (11), stigmasterol (12), ß-sitosterol (13), 6,10,14-trimethyl-2-pentadecanone (14), methyl hexadecanoate(15), methyl octadecanoate (16), and heptacosane (17), whose structures were determined by modern spectroscopic techniques. In which, medicarpin-3-O-glucoside (4) was isolated for the first time from this plant. The antitumour activity of three pure compounds (1), (9) and (10) were tested by using 6 human tumour cell lines. Compound (1) which showed activity in the primary screening was further screened by 36 human tumour cell lines. Compound (1) showed mean IC_{70} value at 11.34 µg/mL and mean IC_{50} value at 7.39 µg/mL in the ovarian tumour cell line.

***Keywords*- *Butea monosperma*; antitumour principle; butein, medicarpin-3-O- glucoside**

I. INTRODUCTION

The plant *Butea monosperma* (Lam.) Kuntze (Syn. *Butea frondosa*; Family Fabaceae) is known as 'Pauk' in Myanmar. In Myanmar Traditional Medicine Hospital it is used as an antiviral and antimalarial remedy and applied for the treatment of hepatitis [1]. It is a moderate sized deciduous tree which is widely distributed throughout Myanmar, India, and Ceylon. This tree is also called 'Flame of the Forest' and Bastard Teak [2]. Its reported chemical constituents contained triterpene, butein, butin, isobutrin, coreopsin, isocoreopsin, sulphurein, monospermoside and isomonospermoside, chalcones, aurones, flavonoids (palasitrin, prunetin) and steroids and reported pharmacological properties included anthelmintic, anticonceptive, anticonvulsive, antidiabetic, antidiarrhoeal, antiestrogenic and antifertility, antiinflammatory, antimicrobial, antifungal, antibacterial, antistress, chemopreventive, haemaggultinating, heap-toprotective, radical scavenging, thyroid inhibitory, and hypoglycemic effects and wound healing activities [3-6].

The flower sample was collected from Yangon University campus and was identified by authorized botanist of Department of Botany, University of Yangon. (Figure 1). The structure elucidation of these compounds was carried out by ^{1}H, ^{13}C, H-H COSY, HSQC, HMBC and mass spectrometry and comparison with literature value and here we wish to report the anticancer activity of three isolated pure compounds from *B. monosperma* and the structure determination of compound (**4**) which was isolated for the first time from this plant and spectroscopic data of isolated chalcones (**1, 3, 10**), flavanones (**2, 7, 8, 9**) and aurone (**6**).

Figure 1. *Butea monosperma* plant

II. MATERIALS AND METHODS

A. General experimental techniques

^{1}H (300 MHz), ^{13}C NMR (75.5 MHz) NMR spectra were measured on a Bruker AMX 300 spectrometer. ESI mass spectra were recorded on a LCQ Finngan Mass Spectrometer,

HR-ESI mass spectra were recorded on APEX IV, 7T, FT-ICR MS Bruker Daltonik. GC-MS were measured on a TRACE GC-MS ThermoFinnigan mass spectrometer. Chromatography was carried out on silica gel (230 – 400 mesh). Thin layer chromatography (TLC) were performed on Polygram SIL G/UV254 (Macherey-Nagel & Co.). R_f values were measured on Polygram SIL G/UV254 (Macherey-Nagel & Co.).

B. Human tumour cell lines and cell proliferation assay

A modified propidium iodide assay was used to assess the effects of the compounds on the growth of the human tumour cell lines. Cells were treated concentration-dependent with the test compounds and surviving cells were stained with a fluorescence dye. Details of the test procedure have been described in [7]. Twenty-two cell lines of the Oncotest cell line collection were used. These cell lines were established from human tumour xenografts growing on nude mice [8, 9]. The other cell lines were kindly provided by the US National Cancer Institute (Bethesda, MD) or were supplied by the ATCC (Rockville, MD).

C. Plant material

B. monosperma (Fabaceae) was collected in Yangon University Campus, Yangon Myanmar, and a voucher specimen, has been deposited in the Herbarium of the Department of Botany, Yangon University.

D. Extraction and isolation

The air-dried flowers of *B. monosperma* (350 g) were extracted with ethonol (6 x 1 L) at room temperature for 3 days. The extract (20.57 g) was dissolved in water and partitationed with cyclohexane, ethylacetate and *n*-butenol successively. From the cyclohexane fraction compound (**11**) to (**17**) were identified by using GC-MS. Ethylacetate fraction (5.97 g) was subjected to silica gel column using dichloromethane/methanol gradient gave compound (**1**) (40 mg) (**2**) (50 mg), (**3**) (170 mg), (**4**) (6 mg), (**5**) (20 mg). The *n*-butanol fraction was subjected to silica gel column using the same solvent gradient like ethyl acetate fraction delivered compounds (**6**) (25 mg), (**7**) (150 mg), (**8**) (130 mg), (**9**) (0.2 g) and (**10**) (0.3 g). Identification of these compounds was carried out by modern spectrometry.

Medicarpin-3-O-glucoside (**4**): Compound (**4**) was obtained as a powder, $C_{22}H_{24}O_9$, $R_{f'}$ = 0.50 (DCM/20% MeOH) **^{1}H-NMR** ([D_6] DMSO, 300 MHz): δ = 7.38 (d, *J* = 8.7 Hz, 1H, 1-H), 7.25 (d, *J* = 8.1 Hz, 1H, 7-H), 6.72 (dd, *J* = 2.5, 8.5 Hz, 1H, 2-H), 6.46 (d, *J* = 2.3 Hz, 1H, 4-H), 6.45 (dd, *J* = 2.3, 8.1 Hz, 1H, 8-H), 6.42 (d, *J* = 2.1 Hz, 1H, 10-H), 5.60 (d, *J* = 6.2 Hz, 1H, 11a-H), 4.84 (d, *J* = 7.3 Hz, 1H, 1'-H), 4.28 (dd, *J* = 9.4, 5.5 Hz, 1H, 6-H_{eq}), 3.70 (s, 3H, 9-OCH_3), 3.66 (m,1H, 6a-H), 3.64 (m, 1H, 6-H_{ax}), 3.65 (m, 1H, 6'-Ha), 3.49 (dd, *J* = 5.5, 11.6 Hz,1H, 6'-Hb), 3.40 (m, 5'-H), 3.22 (m, 3'-H), 3.19 (m, 2'-H), 3.10 (m, 4'-H). (+)- ESI MS: *m/z* (%) = 455.2 $[M+Na]^+$ (18), 886.9 $[2M+Na]^+$ (100), (+)- **ESI-HRMS**, 433.14930 $[M+H]^+$ (calcd.433.14982 for $C_{22}H_{25}O_9$). **^{13}C NMR** ([D_6] DMSO, 125 MHz): δ = 160.5 (C_q, C-9), 160.2 (C_q, C-10a), 158.4 (C_q, C-3), 156.1 (C_q, C-4a), 131.8 (=CH, C-1), 125.1 (=CH, C-7), 119.1 (C_q, C-6b), 114.1 (C_q, C-11b), 110.4 (=CH, C-2), 106.1 (=CH, C-8), 104.0 (=CH, C-4), 100.3 (CH, C-1'), 102.5 (=CH, C-8), 96.3 (CH, C-10), 77.7 (CH, C-11a), 77.0 (CH, C-5'), 76.5(CH, C-3'), 73.1 (CH, C-2'), 69.6 (CH, C-4'), 65.9 (CH_2, C-6), 60.6 (CH_2, C-6'), 55.2 (OCH_3, C-9-OCH_3).

Butein (1): yellow crystal, $C_{15}H_{12}O_5$, $R_{f'}$ = 0.65 (DCM/20% MeOH) **^{1}H-NMR** (CD_3OD, 300MHz): δ = 7.94 (d, *J* = 9.0 Hz, 1H, 6'-H), 7.72 (d, *J* = 15.3 Hz, 1H, α-H), 7.53 (d, *J* = 15.3 Hz, 1H, β-H), , 7.18 (d, *J* = 2.0Hz, 1H, 2-H), 7.11 (dd, *J* = 2.1, 8.2 Hz, 1H, 6-H), 6.81 (d, *J* = 8.2 Hz, 1H, 5-H), 6.41 (dd, *J* = 2.4, 8.9 Hz, 1H, 5'-H), 6.28 (d, *J* = 2.4 Hz, 1H, 3'-H). (-)- **ESIMS**: m/z (%) = 271.1$[M-H]^-$ (96), 544.9 $[2M-H]^-$ (100). **^{13}C NMR** (CD_3OD, 75 MHz) data were shown in Table I.

Butin (2): yellow crystal, $C_{21}H_{22}O_{10}$, $R_{f'}$ = 0.65 (DCM/20% MeOH) **^{1}H-NMR** (CD_3OD, 300MHz): δ = 7.71 (d, *J* = 8.7 Hz, 1H, 5-H), 6.92 (d, *J* = 0.5 Hz, 1H, 2'-H), 6.79 (d, *J* = 8.4 Hz, 1H, 5'-H), 6.77 (dd, *J* = 0.7, 8.1 Hz, 1H, 6'H), 6.48 (dd, *J* = 2.2, 8.7 Hz, 1H, 6-H), 6.35 (d, *J* = 2.2 Hz, 1H, 8-H), 5.31 (dd, *J* = 3.0, 12.9 Hz, 1H, 2-H), 3.00 (dd, *J* = 12.9, 16.9 Hz,1H, 3α-H), 2.68 (dd, *J* = 3.0, 16.9 Hz, 1H, 3β-H). **(+)- ESI MS**: *m/z* (%) = 273.2 $[M+H]^+$ (100), 566.8 $[2M+Na]^+$ (20). **^{13}C NMR** (CD_3OD, 75 MHz) data were shown in Table (2). The **HMBC** spectrum of compound (**2**) showed the following key correlations: H-2' →C-2 and C-6'; H-5'→C-1',C-3' and C-4'; H-6'→C-1',C-2' and C-2; H-3β →C-2, C-1' and C=O; H-3α→ C=O; H-5→C-9, C-7 and C=O; H-6→ C-8 and C-10; H-8→ C-6, C-10, C-7, C-9.

Monospermoside (3): yellow crystal, $C_{21}H_{22}O_{10}$, $R_{f'}$ = 0.52 (DCM/20% MeOH) **^{1}H-NMR** ([D_6] DMSO, 300MHz): δ = 13.56 (s, 1H, 2'-OH), 8.11 (d, *J* = 9.0 Hz, 1H, 6'-H), 7.74 (d, *J* = 15.3 Hz, 1H, α-H), 7.69 (d, *J* = 2.1Hz, 1H, 2-H), 7.67 (d, *J* = 15.3 Hz, 1H, β-H), 7.40(dd, *J* = 2.0, 8.3 Hz, 1H, 6-H), 6.90 (d, *J* = 8.2 Hz, 1H, 5-H), 6.42 (dd, *J* = 2.3, 8.9 Hz, 1H, 5'-H), 6.29 (d, *J* = 2.3 Hz, 1H, 3'-H), 4.85 (d, *J* = 7.3Hz, 1H, 1''-H), 7.69 (d, *J* = 2.1Hz, 1H, 2-H), 5.06 (br s, 2H, sugar-OH), 4.77 (br s, 1H, sugar-OH). **(+)- ESI MS**: *m/z* (%) = 435.0 $[M+H]^+$ (38), 457.2 $[M+Na]^+$ (100), **(-)- ESI MS**: *m/z* (%) = 433.2 $[M-H]^-$ (100). The **HMBC** spectrum of compound (**3**) showed the following key correlations: H-2 →C-1,C-3, C-4, C-6 and C-β; H-5 →C-1,C-3 and C-4; H-6→C-2,C-5, C-4 and C-β; H-1'' →C-3; H-β→ C-6 and C=O; H-α→C-1 and C=O; H-3'→ C-1', C-2', C-4' and C-5'; H-5'→ C-1', C-3'; OH-2'→ C-1', C-2' and C-3'; H-6'→ C-4' and C=O.

Sulphurein (6): red powder, $C_{21}H_{20}O_{10}$, $R_{f'}$ = 0.55 (DCM/20% MeOH) **1H-NMR** (CD_3OD, 300MHz): δ = 7.66 (d, *J* = 8.5 Hz, 1H, 5-H), 7.47 (d, *J* = 2.06 Hz, 1H, 2'-H), 7.21 (dd, *J* = 2.1, 8.3 Hz, 1H, 6'-H), 7.09 (d, *J* = 1.3 Hz, 1H, 8H), 6.94 (dd, *J* = 1.8, 8.5Hz, 1H, 6-H), 6.81 (s, 1H, 2-H), 6.67 (d, *J* = 8.3 Hz, 1H, 5'-H), 5.11 (d, *J* = 7.1 Hz, 1H, CH), 3.94 (dd, *J* = 1.9, 12.9 Hz, 1H, 6a'-H), 3.37 (dd, *J* = 5.4, 12.1 Hz, 1H, 5'-H), 3.52 (d, *J* = 9.4 Hz, 1H, 6b'-H), 3.52 (d, *J* = 4.1 Hz, 1H, 3'-H), 3.51 (d, 3.2, 1H, 4'-H), 3.44 (d, *J* = 9.2 Hz, 1H, 2'-H). (+)- ESI MS: m/z (%) = 433.2 [M+H]+ (5), 887.0 [2M+Na]+ (100), 1318.8 [3M+Na]+ (27), (-)- **ESI MS**: m/z (%) = 431.1[M-H]- (100), 863.1[2M-H]- (97). **^{13}C NMR** ([D_6] DMSO, 125 MHz): δ = 183.7 (Cq, C-4), 168.1 (Cq, C-8a), 166.0 (Cq, C-7), 159.6 (Cq, C-3), 149.4 (Cq, C-4'), 146.0 (Cq, C-3'), 129.1 (Cq, C-1'), 125.9 (=CH, C-5), 120.9 (=CH, C-6'), 118.9 (=CH, C-

5'), 118.0 (=CH, C-2'), 120.9 (=CH, C-6'), 117.8 (Cq, C-4a), 116.9 (=CH, C-6), 114.5 (=CH, C-2), 101.7 (CH, C-1''), 78.3 (CH, C-4''), 77.8 (CH, C-5''), 74.8 (CH, C-2''), 71.2 (CH, C-3''), 62.4 (CH_2, C-6'').

Isomonospermoside (7): yellow crystal, $C_{21}H_{22}O_{10}$, R_f = 0.45 (DCM/20% MeOH) **^{1}H-NMR** ([D_6] DMSO, 300MHz): δ = 10.58(br s, 1H, OH), 8.71 (br s, 1H, OH), 7.64 (d, *J* = 8.6 Hz, 1H, 5-H), 7.28 (d, *J* = 1.9 Hz, 1H, 2'-H), 7.03 (dd, *J* = 1.9, 8.3 Hz, 1H, 6'-H), 6.84 (d, *J* = 8.2 Hz, 1H, 5'H), 6.50 (dd, *J* = 2.2, 8.6 Hz, 1H, 6-H), 6.35 (d, *J* = 2.2 Hz, 1H, 8-H), 5.41(dd, *J* = 2.8, 12.6 Hz, 1H, 2-H), 5.04 (br s, 1H, OH), 4.98 (br s, 1H, OH), 4.71 (d, *J* = 7.3Hz, 1H, 1''-H), 4.53 (br s,1H, sugar OH), 4.05 (br s, 1H, sugar OH), 3.71 (d, 11.2, 1H, 6''-H), 3.49 (dd, *J* = 5.5, 11.6 Hz,1H, sugar-H), 3.10 (dd, *J* = 12.6, 16.8 Hz,1H, 3a-H), 2.66 (dd, *J* = 2.9, 16.8 Hz,1H, 3b-H). **(+)- ESI MS**: *m/z* (%) = 457.2 $[M+Na]^+$ (14), 890.9 $[2M+Na]^+$ (100), **(-)- ESI MS**: *m/z* (%) = 433.2 $[M-H]^-$ (100), 866.9 $[2M-H]^-$ (78). The **HMBC** spectrum of compound (**7**) showed the following key correlations: H-2' →C-2, C-3', C-4' and C-6'; H-5'→C-1' and C-3'; H-6'→ C-2' and C-2; H-3β →C-2, C-1' and C=O; H-3α→ C=O; H-5→ C-7 and C=O; H-6→ C-8 and C-10; H-8→ C-6 and C-10; H-1''→C-3'.

Isocoreopsin (8): yellow crystal, $C_{21}H_{22}O_{10}$, R_f = 0.45 (DCM/20% MeOH) **^{1}H NMR** ([D_6] DMSO, 300MHz): δ = 10.58 (br s, 1H, OH), 8.71 (br s, 1H, OH), 7.64 (d, *J* = 8.6 Hz, 1H, 5-H), 7.28 (d, *J* = 1.9 Hz, 1H, 2'-H), 7.03 (dd, *J* = 1.9, 8.3 Hz, 1H, 6'-H), 6.84 (d, *J* = 8.2 Hz, 1H, 5'H), 6.50 (dd, *J* = 2.2, 8.6 Hz, 1H, 6-H), 6.35 (d, *J* = 2.2 Hz, 1H, 8-H), 5.41(dd, *J* = 2.8, 12.6 Hz, 1H, 2-H), 5.04 (br s, 1H, OH), 4.98 (br s, 1H, OH), 4.71 (d, *J* = 7.3Hz, 1H, 1''-H), 4.53 (br s,1H, sugar OH), 4.05 (br s, 1H, sugar OH), 3.71 (d, 11.2, 1H, 6''-H), 3.49 (dd, *J* = 5.5, 11.6 Hz,1H, sugar-H), 3.10 (dd, *J* = 12.6, 16.8 Hz,1H, 3a-H), 2.66 (dd, *J* = 2.9, 16.8 Hz,1H, 3b-H). **(+)- ESI MS**: *m/z* (%) = 457.2 $[M+Na]^+$ (14), 890.9 $[2M+Na]^+$ (100), **(-)- ESI MS**: *m/z* (%) = 433.2 $[M-H]^-$ (100), 866.9 $[2M-H]^-$ (78).

Butrin (9): yellow crystals, $C_{27}H_{32}O_{15}$, *Rf*' = 0.25 (DCM/20% MeOH) 1H NMR ([D_6] DMSO, 300MHz): δ = 7.72 (d, *J* = 8.7 Hz, 1H, 5-H), 7.30 (d, *J* = 2.1 Hz, 1H, 2'-H), 7.04 (dd, *J* = 2.0, 8.2 Hz, 1H, 6'-H), 6.85 (d, *J* = 8.2 Hz, 1H, 5'H), 6.72 (dd, *J* = 2.2, 8.7 Hz, 1H, 6-H), 6.67 (d, J = 2.2 Hz, 1H, 8-H), 5.47 (dd, J = 2.7, 12.7 Hz, 1H, 2-H), 5.30 (d, 3.5 Hz, 1H, OH), 5.03 (br s, sugar OH), 4.97 (d, J = 7.3 Hz, 1H, 1''-H), 4.71 (d, J = 7.3 Hz, 1H, 1'''-H), 4.54 (t, J = 5.1, 1H, sugar OH), 4.50 (t, J = 6.1, 1H, sugar OH), 3.68 (t, J = 7.7, 2H, 6'' and 6'''-H), 3.19 (dd, J = 13.0, 17.0 Hz, 1H, 3a-H), 2.69 (dd, J = 13.0, 17.0 Hz, 1H, 3b-H). 13C NMR ([D6] DMSO, 75 MHz) data were shown in Table 1. (+)- ESI MS: m/z (%) = 619 [M+Na]+ (100), 1215 [2M+Na]+ (46), (-)- ESI MS: m/z (%) = 595 [M-H]- (100), 866.9. The HMBC spectrum of compound (9) showed the following key correlations: H-2' →C-2, C-3', C-4' and C-6'; H-5'→C-1', C-3'and C-4'; H-6'→ C-2' and C-2;H-3β →C-2 and C=O; H-5→C=O; H-6→ C-8 and C-10; H-8→ C-6, C-10 and C-9; H-1''→C-7; H-1'''→C-3'.

Isobutrin (10): yellow crystal, $C_{27}H_{32}O_{15}$, Rf = 0.32 (DCM/20% MeOH) ^{1}H-NMR ([D_6] DMSO, 300MHz): δ = 13.43 (s, 1H, 2'-OH), 8.06 (d, *J* = 9.1 Hz, 1H, 6'-H), 7.77 (d, *J* = 15.3 Hz, 1H, α-H), 7.69 (d, *J* = 1.9 Hz, 1H, 2-H), 7.61 (d, *J* = 15.3 Hz, 1H, β-H), 7.29 (dd, *J* = 1.9, 8.4 Hz, 1H, 6-H), 6.88 (d, *J* = 8.2 Hz, 1H, 5-H), 6.67 (dd, *J* = 2.4, 9.0 Hz, 1H, 5'-H), 5.59 (d, *J* = 2.4 Hz, 1H, 3'-H), 5.30 (br s, sugar-OH), 5.03 (d, *J* = 7.3 Hz, 1H, 1'''-H), 4.89 (d, *J* = 7.2 Hz, 1H, 1''-H), 4.77 (br s, sugar-OH). ^{13}C NMR ([D_6] DMSO, 75 MHz) data were shown in Table 1. (+)- ESI MS: m/z (%) = 597.0 $[M+H]^+$ (38), 619.2 $[M+Na]^+$ (100), (-)- **ESI MS**: m/z (%) = 595 $[M-H]^-$ (100). The **HMBC** spectrum of compound (**10**) showed the following key correlations: H-2 →C-3, C-4, C-6 and C-β; H-5 →C-1,C-3 and C-4; H-6→C-1,C-2, C-4 and C-β; H-β→ C-2, C-6 and C=O; H-α→C-1, C-β and C=O; H-3'→ C-1', C-2', C-4' and C-5'; H-5'→ C-1', C-3'; H-6'→C-2', C-4' and C=O; H-1'' →C-3; H-1''' →C-4'.

III. RESULTS AND DISCUSSION

From the ethylacetate fraction of the flowers of *B. monosperma* was separated and purified by column chromatography on silica gel. Compounds (**1-5**) from this fraction and compound (**6-10**) from *n*-butanol fraction were obtained and their structures were identified by modern spectroscopic techniques. Compound (**4**) gave a molecular formula $C_{22}H_{24}O_9$ by high resolution ESI-MS. The ^{1}H NMR spectrum of **4** exhibited a set of proton signals [3.64 (m, 1H, 6-H_{ax}), 4.28 (dd, *J* = 9.4, 5.5 Hz, 1H, 6-H_{eq}), 3.66 (m,1H, 6a-H), 5.60 (d, *J* = 6.2 Hz, 1H, 11a-H)] characteristics of a pterocapan skeleton. The spectrum also showed two ABX-type aromatic proton signals at δ 7.38 (d, *J* = 8.7 Hz, 1H), 6.72 (dd, *J* = 2.5, 8.5 Hz, 1H) and 6.46 (d, *J* = 2.3 Hz, 1H) due to A ring protons, and δ 7.25 (d, *J* = 8.1 Hz, 1H), 6.45 (dd, *J* = 2.3, 8.1 Hz, 1H) and 6.42 (d, *J* = 2.1 Hz, 1H) due to B ring protons, a methoxy proton signals at δ 3.70 (s, 3H) and an anomeric proton signal at δ 4.84 (d, *J* = 7.3 Hz, 1H) suggesting a β-D-glucopyranoside. The ^{13}C NMR spectrum of **4** showed 21

signals and one signal was submerged under solvent peak which was confirmed in HSQC spectrum. The positions of methoxy and β-D-glucopyranosyl groups in **4** were determined from HMBC spectra as shown in Figure 2. Thus, the structure of compound **4** was determined as Medicarpin-3-O-glucoside**.**

The four flavanones (**2, 7, 8, 9**) were isolated as pale yellow powder. The TLC spots turned dark yellow with anisaldehyde/sulphuric acid. With ammonia they changed to orange. The ESI mass spectra of (**2, 7, 8, 9**) confirmed their masses and all the ^{1}H and ^{13}C NMR spectra agreed with chalcone skeleton. Comparison of ^{13}C NMR data was shown in Table I. The ^{1}H and ^{13}C NMR assignments were confirmed by HSQC, ^{1}H ^{1}H COSY and MHBC experiments. The structure of (**8**) was identified by comparison with (**7**).

The three chalcones (**1, 3, 10**) were isolated as yellow powder. On fuming with ammonia, the colour changed to orange red suggesting as chalcones. All three ^{1}H NMR spectra indicated the presence of two trans-olefinic protons. ^{13}C NMR data comparison were shown in Table II.

Compound (6) was obtained as a red powder and the structure was identified as sulphurein by using ESI MS, 1H and 13C NMR and NOE spectra. The structures of isolated compounds were shown in Figures 2-4.

A. Antitumour Activity

Three pure compounds (**1**, **9** and **10**) were tested for the antitumour activity by using six different human tumour cell lines at two different concentrations of 1.0 and 10.0 µg/mL.

TABLE I. ^{13}C NMR SPECTRAL ASSIGNMENTS OF FOUR FLAVANONES (**2**, **7**, **8**, **9**) (75.5 MHZ, ([D_6] DMSO)

C	9	7	8	2
2	79.2	78.8	79.0	78.9
3	43.0	43.0	43.0	43.2
4	190.4	189.9	190.0	189.9
5	127.9	128.3	128.3	128.2
6	110.9	110.4	110.5	110.4
7	163.5	164.6	164.6	164.6
8	103.6	102.5	102.6	102.5
4a	115.3	113.5	113.5	114.2
8a	162.8	163.0	163.1	163.0
1′	129.7	130.0	129.9	129.9
2′	115.5	115.6	115.4	113.5
3′	145.1	145.1	145.0	145.5
4′	147.0	147.0	147.0	145.1
5′	115.7	115.7	115.7	117.7
6′	121.7	121.5	121.5	115.3
1″	102.0	102.3	102.0	
2″	73.3	73.3	73.3	
3″	77.2	77.1	77.1	
4″	69.9	69.8	69.9	
5″	76.4	75.9	76.0	
6″	60.8	60.6	60.7	
1‴	99.8			
2‴	73.1			
3‴	77.0			
4‴	69.5			
5‴	76.0			
6‴	60.6			

TABLE II. ^{3}C NMR SPECTRAL ASSIGNMENTS OF THREE CHALCONES (**1, 3,10**) (75 MHZ, ([D_6] DMSO

C	10	3	1
1	128.5	126.4	126.2
2	118.1	116.4	115.7
3	147.7	145.7	145.6
4	157.5	149.8	148.9
5	117.5	116.2	115.7
6	127.3	125.5	122.2
α	118.9	117.9	117.3
β	146.2	144.3	144.6
C=O	193.4	191.4	191.3
1′	116.6	113.0	112.9
2′	166.8	165.0	164.9
3′	105.1	102.6	102.5
4′	165.1	165.8	165.6
5′	109.4	108.2	108.1
6′	133.2	132.8	132.6
1″	104.2	102.1	
2″	74.9	73.4	
3″	77.8	76.0	
4″	71.7	70.3	
5″	78.7	77.4	
6″	62.7	61.1	
1‴	101.3		
2‴	74.7		
3‴	77.6		
4‴	71.7		
5‴	78.3		
6‴	62.3		

TABLE III. CANCER CELL LINES NAMES AND ACTIVITY FOR BUTEIN (**1**)

No.	Name of cancer cell organ	Names of cell line	Activity		
			IC_{50} µg/mL	IC_{70} µg/mL	IC_{90} µg/mL
1	Bladder	BXF1218L	5.586	9.484	16.102
		BXFT24	5.586	9.484	16.102
2	Central nervous system	CNXF498NL	17.508	>10.000	>10.000
		CNXFSF268	3.455	6.235	11.253
3	Colon	CXFHCT116	3.334	6.772	13.755
		CXFHT29	>10.000	>10.000	>10.000
4	Gastric	GXF251L	4.216	9.597	21.841
5	Head & Neck	HNXF536L	10.985	28.117	>10.000
6	Lung	LXF1121L	11.522	23.400	>10.000
		LXF289L	23.194	>10.000	>10.000
		LXF526L	10.284	18.034	>10.000
		LXF529L	3.486	7.609	16.608
		LXF629L	>10.000	>10.000	>10.000
		LXFH460	>10.000	>10.000	>10.000
7	Mammary	MAXF401NL	5.000	7.819	12.228
		MAXFMCF7	>10.000	>10.000	>10.000
8	Melanomas	MEXF276L	9.582	22.482	>10.000
		MEXF394NL	6.716	11.859	20.940
		MEXF462NL	3.694	6.883	12.826
		MEXF514L	5.498	10.000	18.186
		MEXF520L	10.471	26.302	>10.000
9	Ovarian	OVXF1619L	2.430	4.692	9.060
		OVXF899L	>10.000	>10.000	>10.000
		OVXFOVCAR3	6.476	15.441	>10.000
10	Pancreatic	PAXF1657L	23.101	>10.000	>10.000
		PAXFPANC1	6.105	13.894	>10.000
11	Prostate	PRXF22RV1	16.015	>10.000	>10.000
		PRXFDU145	>10.000	>10.000	>10.000
		PRXFLNCAP	5.772	9.741	16.440
		PRXFPC3M	5.623	9.490	16.015
12	Pleuramesothelioma	PXF1752L	5.080	8.733	15.013
13	Renal	RXF1781L	5.011	8.912	15.848
		RXF393NL	>10.000	>10.000	>10.000
		RXF486L	8.220	21.899	>10.000
		RXF944L	5.584	9.726	16.940
14	Uterus	UXF1138L	7.060	12.061	20.604
Mean		n=25	7.385	11.338	15.501

TABLE IV. *IN VITRO* ANTITUMOUR ACTIVITY OF COMPOUND (**1**) IN HUMAN TUMOUR CELL LINES

No.	Compound	Mean ICs [µg/mL]		Tumour selectivity	
		IC_{50}	IC_{70}	Active*/total	%
1	**1**	7.39	11.34	1/36	3%

* individual IC_{70} < 1/2 mean IC_{70}

TABLE V. SELECTIVE ANTITUMOUR ACTIVITY OF COMPOUND (**1**) IN HUMAN TUMOUR CELL LINE

Compound	Mean ICs [µg/mL]		Result			
			Tumor selectivity			
	IC_{50}	IC_{70}	Active*/ total	%	Sensitive cell line*	Most sensitive cell line**
1	7.39	11.34	1/36	3	OVXF161L	-

* individual IC_{70}<1/2 mean IC_{70} ** individual IC_{70}<1/3 mean IC_{70}

Only compound (**1**) which showed activity in the primary screening was further screened by 36 human tumour cell lines using five different concentrations. The screening comprised cell lines derived from bladder, central nervous system, colon, gastric, head & neck, lung, mammary, ovarian, pancreatic, prostate, renal and uterus cancers, as well as cell lines established from melanomas and pleuramesothelioma.

The antitumour activity of compound **1** was indicated in the Table 1-3. The compound **1** exhibited mean IC_{50} value of 7.39 µg/mL and mean IC_{70} value of 11.34 µg/mL. The compound **1** effected pronounced activity in ovarian cancer cell line OVXF1619L.

The major compound Butein (**1**) isolated from *B. monosperma* exhibited pronounced antitumour activity and should be used for the treatment of ovarian cancer.

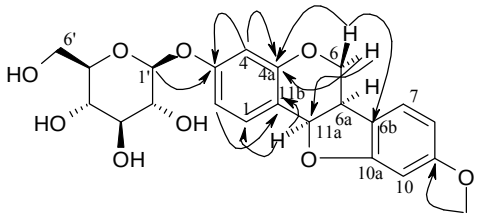
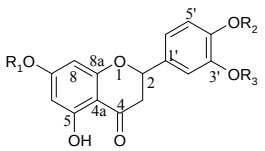
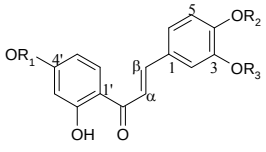


Figure 2. Significant HMBC correlations of **4**



Butin (**2**); R_1=R_2=R_3=H
Isomonospermoside (**7**);
R_3= Glu, R_1= R_2= H
butrin (**9**); R_1= Glu (1''), R_2= H, R_3= Glu (1'''),
Isocoreopsin (**8**);
R_1= Glu, R_2 = R_3= H



Butein(**1**);R_1=R_2=R_3=H
monospermoside (**3**);
R_3= Glu, R_1= R_2= H
isobutrin (**10**);
R_1= Glu (1'''), R_2 = H,
R_3 = Glu (1'')

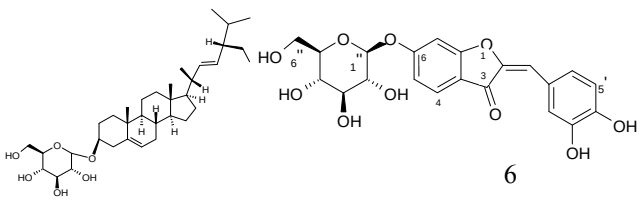


Figure 3. Structures of compounds (**1-10**) isolated from *B. monosperma*

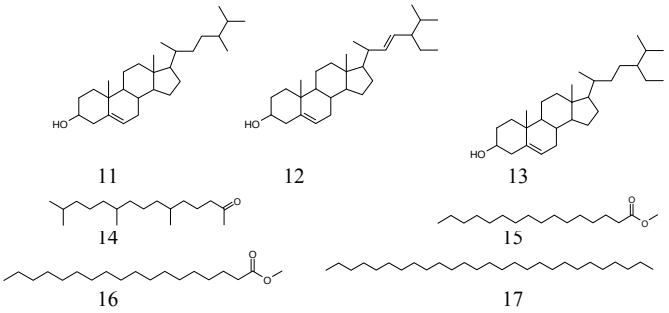


Figure 4. Structures of compounds (**11-17**) identified by GC-MS

IV. CONCLUSION

In conclusion, The antitumour activity and selectivity of the pure compound Butein (**1**) isolated from *B. monosperma* was reported for the first time. The compound (**1**) (IC_{50} = 7.39 µg/mL and IC_{70} = 11.34 µg/mL) showed sensitive to ovarian cancer cell line OVXF1619L. Therefore, our research findings could be used for developing Myanmar traditional medicine formulations, using plant source for the treatment of cancer which can ultimately be beneficial for the human beings.

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