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Antioxidative and cytotoxic activities of crude and isolated compounds of *P. Lateriflora* (bl.) King

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Abstract:

This study aimed to isolate compounds from *Polyalthia lateriflora* (Bl.) King stem bark, and to test the crude extracts and the isolated compounds for their antioxidant and cytotoxic activities. Three different solvents were used to prepare the crude extracts; hexane, dichloromethane (DCM) and methanol. Phytochemical investigation of the crude extracts has led to the isolation of four oxoaporphine alkaloids (O-methylmoschatoline, atherospermidine, lysicamine, and liriodenine) and two steroids (stigmasterol and lupeol). The chemical structure of the compounds was elucidated using ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, and mass spectral analysis. All the crude extracts and isolated compounds were tested for their *in vitro* DPPH radicals scavenging activity and cytotoxicity against MCF-7 breast cancer cells. Methanol extract showed the strongest antioxidant activity with percentage inhibition at 500µg/mL of 89.52±1.81 ($IC_{50,}$ 128.22± 6.73 µg/ml) in comparison with DCM (32.45±8.56), atherospermidine (20.17±9.74) and liriodenine (10.73±0.45), while other extracts were inactive. All the isolated compounds showed cytotoxic against MCF-7 cells, at IC_{50} values in the range of 42-89 µg/ml. Based on thorough literature review, all compounds identified in this study were isolated for the first time from this plant. In particular, lupeol is reported for the first time in the genus *Polyalthia* and possessed the most potent cytotoxic effect compared to other isolated compounds.

Key word: *Polyalthia lateriflora* (Bl.) King, antioxidant, DPPH scavenger, cytotoxicity, MCF-7 cells

1. INTRODUCTION

Polyalthia lateriflora (Bl.) King (KL 5255) is a flowering plant belongs to the genus *Polyalthia*, of the big family Annonaceae (consists of 130 genus and 2300-2500 species) [1,2,3]. According to the phytochemical survey of 'Malaysian Herbarium on Flora of Malaysia' this plant is dominant locally at the area of Hutan Simpan Bukit Kinta, Chemor, Perak. It can grow to reach 12 m tall and 20 cm diameter. Several species of the genus *Polyalthia* have been reported to have various medicinal properties, including antioxidant [4], anticancer [5,6,7], antibacterial [8,9], antifungal [10], antiulcer [11], lipid lowering agent [12], protective effect against oxidative DNA damage [13] and antiplasmodial [14]. Phytochemical studies on *Polyalthia* species indicated the presence of various types of chemical compounds, such as alkaloids (e.g cerasoidine and bidebiline E) [15], terpenes (e.g 16(*R*&*S*)-methoxycleroda-4 (18), 13-dien-15,16-olide) [16], flavones (e.g 3,7-dimethoxy-5-hydroxyflavone, and 5,8-dihydroxy-6,7-dimethoxy flavone) [17], chalcone (e.g 2',4'-dihydroxy-3'-methoxy chalcone, and 2',4'-dihydroxy chalcone) [18] and steroids (e.g. stigmasterol) [19]. To the best of our knowledge, this present study is the first work carried out on *P. lateriflora* stem bark.

2. MATERIALS AND METHODS

2.1. Instruments and Reagents

The ^{1}H, ^{13}C, and 2D (COSY, HMBC, HMQC) NMR studies were carried out using JEOL ECX system at 500 MHz. Aluminum supported silica gel 60 F_{254} plates were used for thin-layer chromatography (TLC), visualized under ultraviolet light (254 and 365 nm), and sprayed using Dragendorff's reagent for alkaloid test and *p*-anisaldehyde reagent for steroid test. Silica gel 60 (200–400 mesh) was used for column chromatography. Materials for antioxidant assay include DPPH and ascorbic acid (Sigma-Aldrich Chemical Co., USA), methanol and DMSO (Merck, Germany). MCF-7 breast cancer cells (ATCC® HTB-22™) were used in cytotoxicity assay.

2.2. Plant materials

P. lateriflora (Bl) king stem bark were collected from Hutan Simpan, Bukit Kinta, Chemor, Perak. The species was taxonomically identified by the phytochemical group of Chemistry Department, University of Malaya, Kuala Lumpur, where the voucher specimen was deposited.

2.3. Extraction and isolation

The dried stem bark of *P. lateriflora* (2.0 kg) were crushed in an electric grinder, pulverized into coarse powder and divided into three parts. Each part was transferred into a thimble and inserted into a Soxhlet assembly fitted with a 5L flask. Amount of 4L of hexane, dichloromethane, and methanol were added to each flask respectively, and kept at suitable temperature for 20h. After filtration and drying under low pressure and low temperature of rotary evaporator fitted with a vacuum pump, amount of 50g, 22g and 40g of hexane, DCM and methanol crude extracts were produced respectively.

DCM crude extract (20g) was subjected to silica gel column chromatography, eluted with DCM /MeOH (100:1 to 1:100 v/v) and yielding 52 fractions (Fr.1–Fr.52).

The fractions Fr.1-Fr.18 were subjected to CC on silica gel using DCM/MeOH (100:0 to 95:5 v/v) and afforded 14 fractions. The sub-fractions sFr.10 to sFr.12 were further subjected to CC on silica gel using EA/DCM (15:85 to 40:60 v/v) and produced 14 fractions.

The fractions Fr.5–Fr.9 were subjected to CC on silica gel using DCM/EA (75:25 v/v) and afford three fractions; the third fraction was *O*-methylmoschatoline (1.7mg).

The fractions Fr.21–Fr.26 were subjected to CC on silica gel using DCM/MeOH (100:1 to 86:14 v/v) to afford 45 sub-fractions; sFr.12 and sFr.13 were further subjected to CC on silica gel using DCM/EA (3:1 v/v) and produced 16 fractions; fractions 1 to 8 were atherospermidine (3.1mg), and fraction 16 was lysicamine (2.1mg).

The fractions Fr.27–Fr.38 were subjected to CC on silica gel using DCM/MeOH (100:0 to 95:5 v/v) and afforded 11 sub-fractions; the sFr.7–sFr.10 were subjected to CC on silica gel using EA/hexane (4:1 v/v) and afforded 10 fractions 8 to 10, which were liriodenine (5mg).

The hexane crude extract (25g) was introduced to first column chromatography over silica gel with hexane/EA (100:0 to 50:50 v/v) and DCM/MeOH (75:25 v:v) as eluting solvents. Eluents were collected and yielded 33 fractions.

The fractions Fr.2–Fr.5 were subjected to CC on silica gel using hexane/DCM (100:0 to 50:50 v/v) and afforded 14 sub-fractions. The sub-fraction number 5 (sFr.5) was further subjected to CC on silica gel using hexane/DCM (70:30 v/v) and produced 4 fractions; fraction number 2 and 3 were stigmasterol (7mg).

The fractions Fr.8–Fr.10 were subjected to CC on silica gel using hexane/EA (95:5 to 75:25 v/v) and afforded 9 fractions. The sub-fractions 4 and 5 sFr.4-sFr.5 were further subjected to CC on silica gel using hexane/DCM (75:25 v/v) and produced 4 fractions; fraction number 2 was lupeol (5.3mg).

2.4. DPPH radical scavenging activity

The DPPH assay was based on the 96-well plate assay. A total of 20 µL of the two-fold serially diluted sample was added to 180 µL of DPPH solution (150 µmol L^{-1}) in methanol:water (80:20 v/v). All samples were tested at the concentration range 3.9–500 µg/mL. The microplate was shaken for 60 seconds and left in the dark for 40 minutes at room temperature. Absorbance was measured at 515nm using the microplate reader. Ascorbic acid was used as a standard at 0.39–50 µg/mL. [20]

2.5. Cytotoxic Assay

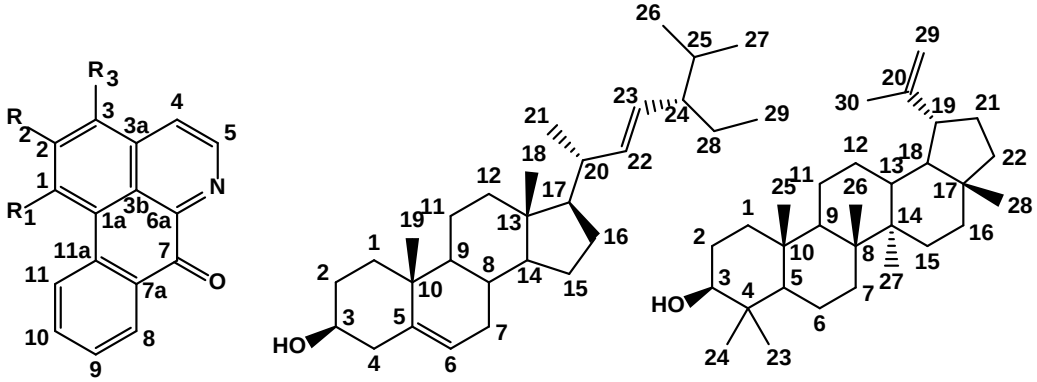
The MCF-7 breast cancer cells were cultured *in-vitro*. Once the cells reached their active stage, they were transferred into 96-well tissue culture plate, at a concentration of $1x10^4$ cells/well with 100µl of cell suspension into each well. The cells were incubated in humidified carbon dioxide (CO_2) incubator for 24 h prior to adding samples and negative control (10µl respectively). The cells were incubated for another 48 h. After that, 10µl of Cell Count Reagent was added to each well. The cells were incubated for another 2 h, followed by absorbance measurement at 450nm using microplate reader. IC_{50} value (concentration of compound that yields 50% less cells compared to the control) was derived from curve-fitting methods [21, 22].

3. Results And Discussion

Six compounds were isolated and identified in this current study (Figure 1). The structure of each isolated compound was elucidated using ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, mass spectral analysis, and by compared with the reported data.

O-methylmoschatoline **(1)**: Orange amorphous; Positive LC-MS: *m/z* 321.1 $[M]^+$, chemical formula: $C_{19}H_{15}NO_4$, molecular weight: 321.3; ^{1}H-NMR ($CDCl_3$ with 1% v/v TMS, 500 MHz) δ (ppm): 9.08 (1H, *d*, *J* = 7.45 Hz, H-11), 8.94 (1H, *d*, *J* = 5.15 Hz, H-5), 8.54 (1H, *d*, *J* = 8 Hz, H-8), 8.19 (1H, *d*, *J* = 5.75 Hz, H-4), 7.73 (1H, *t*, H-10), 7.52 (1H, *t*, H-9), 4.17 (3H, *s*, OCH_3-3), 4.08 (3H, *s*, OCH_3-2), 4.06 (3H, *s*, OCH_3-1); ^{13}C-NMR ($CDCl_3$ with 1% v/v TMS, 125 MHz) δ (ppm): 182.73 (C-7), 156.55 (C-1), 148.53 (C-3), 147.39 (C-2), 145.55 (C-6a), 144.63 (C-5), 134.60 (C-11a), 134.46 (C-10), 131.49 (C-7a), 131.15 (C-3a), 128.98 (C-8), 128.22 (C-9), 127.73 (C-11), 122.89 (C-3b), 119.23 (C-4), 115.70 (C-1a), 61.90 (OCH_3-3), 61.57 (OCH_3-2), 61.09 (OCH_3-1).

Atherospermidine **(2)**: Orange amorphous powder, LC-MS: *m/z* 306.1 $[M]^+$, chemical formula: $C_{18}H_{11}NO_4$, molecular weight: 305.284; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 4.27 (3H, *s*, OCH_3-3), 6.32 (2H, *s*, 1-OCH_2O-2), 8.11 (1H, *d*, *J* = 5.7, H-4) 8.87 (1H, *d*, *J* = 5.75, H-5), 8.45 (1H, *d*, *J* = 8, H-8), 8.49 (1H, *d*, *J* = 6.85, H-11), 7.48 (1H, *t*, H-9), 7.66 (1H, *t*, H-10). ^{13}C-NMR ($CDC1_3$ with 1% v/v TMS, 125 MHz) δ (ppm): 182.58 (C-7), 149.71 (C-l), 144.97 (C-6a), 144.31 (C-5), 136.53 (C-3), 136.26 (C-2), 133.96 (C-10), 133.23 (C-11a), 130.74 (C-3a), 130.61 (C-7a), 128.71 (C-8), 127.65 (C-9), 126.66 (C-11), 122.88 (C-3b), 119.37 (C-4), 102.62 (C-la), 102.36 (1-OCH_2O-2) and 60.22 (OCH_3-3).



	R1	R2	R3
(1)	OCH_3	OCH_3	OCH_3
(2)	OCH_2O		OCH_3
(3)	OCH_3	OCH_3	H
(4)	OCH_2O		H

Figure 1: Structures of compounds 1- 6

Lysicamine **(3)**: Yellow amorphous powder, positive LC-MS: *m/z* 291.1 $[M]^+$, chemical formula: $C_{18}H_{13}NO_3$, molecular weight: 291.3; ^{1}H-NMR ($CDC1_3$ with 1% v/v TMS, 500 MHz) δ (ppm): 9.15 (1H, *d, J* = 7.5 Hz, H-11), 8.87 (1H, *d, J* = 5.5 Hz, H-5) 8.56 (1H, *d, J* = 7.5 Hz, H-8), 7.78 (1H, *d, J* = 5.0Hz, H-4), 7.76 (1H, *t*, H-10), 7.56 (1H, *t*, H-9), 7.20 (1H, *s*, H-3), 4.10 (3H, *s*, OCH_3-l) and 4.02 (3H, *s*, OCH_3-2). ^{13}C-NMR ($CDC1_3$ with 1% v/v TMS, 125 MHz) δ (ppm): 156.92 (C-l), 119.91 (C-la), 152.15 (C-2), 106.55 (C-3), 135.60 (C-3a), 122.27 (C-3b), 123.71 (C-4), 145.13 (C-5), 145.44 (C-6a), 182.82 (C-7), 132.16 (C-7a), 128.98 (C-8), 128.90 (C-9), 134.41 (C-10), 128.53 (C-11), 134.46 (C-11a), 56.32 (OCH_3-l) and 60.76 (OCH_3-2).

Liriodenine **(4)**: Yellow amorphous, positive LC-MS: *m/z* 276.1 $[M]^+$, Chemical Formula: $C_{17}H_{11}NO_3$, molecular weight: 277.28; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.39 (2H, *s*, O-CH_2-O), 7.24 (1H, *s*, H-3), 7.77 (1H, *d, J*= 5.15, H-4), 8.90 (1H, *d, J* = 5.2, H-5), 8.57 (1H, *d, J* = 8, H-8), 7.76 (1H, *t*, H10), 8.66 (1H, *d, J* = 8, H-11), 7.58 (1H, *t*, H-9). ^{13}C-NMR ($CDC1_3$ with 1% v/v TMS, 125 MHz) δ (ppm): 148.10 (C-l), 135.88 (C-la), 151.90 (C-2), 103.33 (C-3), 123.29 (C-3a), 108.12 (C-3b), 124.40 (C-4), 144.83 (C-5), 145.24 (C-6a), 182.48 (C-7), 131.27 (C-7a), 128.68 (C-8), 128.87 (C-9), 134.07 (C-10), 127.44 (C-11), 132.92 (C-11a) and 102.62 (1-O-CH_2O-2).

Stigmasterol **(5)**: White steroid, Positive LC-MS: *m/z* 412.4 $[M]^+$, chemical formula: $C_{29}H_{48}O$, molecular weight: 412.691; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 0.64-0.90 (*m*, 15H), 0.91-0.98 (*m*, 2H), 0.99-1.02 (*m*, 6H), 1.04-1.11 (*m*, 1H), 1.13-1.19 (*m*, 2H), 1.25-1.29 (*m*, 2H), 1.49-1.56 (*m*, 8H), 1.81-1.88 (*m*, 3H), 1.94-2.06 (*m*, 3H), 2.21-2.32 (*m*, 2H), 3.49-3.55 (*m*, 1H), 4.98-5.03 (*m*, 1H), 5.12-5.17 (*m*, 1H), 5.34-5.35 (*m*, 1H). ^{13}C-NMR ($CDCl_3$ with 1% v/v TMS, 125 MHz) δ (ppm): 37.32 (C-1), 31.72 (C-2), 71.90 (C-3), 42.29 (C-4), 140.82 (C-5), 121.82 (C-6), 31.97 (C-7), 31.97 (C-8),50.19 (C-9), 36.58 (C-10), 21.20 (C-11), 39.74 (C-12), 42.36 (C-13), 56.83 (C-14), 24.38 (C-15), 29.03 (C-16), 56.00 (C-17), 12.13 (C-18), 19.49 (C-19), 40.62 (C-20), 21.15 (C-21), 138.42 (C-22), 129.33 (C-23), 51.32 (C-24), 32.50 (C-25), 21.30 (C-26), 19.06 (C-27), 25.51 (C-28), 12.36 (C-29).

Lupeol **(6)**: White steroid, Chemical Formula: $C_{30}H_{50}O$, Molecular Weight: 426; ^{1}H-NMR ($CDCI_3$ with 1% v/v TMS, 500 MHz) δ (ppm): 0.88, 1.61 (1H each, *m*, H-1), 1.50, 1.57 (1H each, *m*, H-2), 3.18 (1H, *m*, H-3), 0.65 (1H, *d, J* = 9.7 Hz, H-5), 1.34, 1.52 (1H each, *m*, H-6), 1.44 (1H, *m*, H- 7), 1.27 (1H, *d, J* = 3.4 Hz, H-9), 1.21, 1.40 (1H each, *m*, H-11), 1.06, 1.69 (1H each, *m*, H-12), 1.61 (1H, *m*, H-13), 1.03, 1.71 (1H each, *m*, H-15), 1.38, 1.45 (1H each, *m*, H-16), 1.31 (1H, *t*, H-18), 2.39 (1H, *m*, H-19), 1.28, 1.90 (1H each, *m*, H-21), 1.16, 1.40 (1H each, *m*, H-22), 0.95 (3H, *s*, H-23), 0.74 (1H, *m*, H-24), 0.81 (1H, *m*, H-25), 1.01 (3H, *m*, H-26), 0.92 (3H, *m*, H-27), 0.77 (3H, *m*, H-28), 4.55 (1H, *s*, H-29), 4.66 (1H, *s*, H-29) and 1.66 (3H, *s*, H-30). ^{13}CNMR ($CDCl_3$ with 1% v/v TMS, 125 MHz) δ (ppm): 38.77 (C-1), 27.51 (C-2),78.09 (C-3), 38.94 (C-4), 55.35 (C-5), 18.39 (C-6), 34.33 (C-7), 40.89 (C-8), 50.48 (C-9), 37.23 (C-10), 20.99 (C-11), 25.18 (C-12), 38.10 (C-13), 42.90 (C-14), 27.47 (C-15), 35.65 (C-16), 43.08 (C-17), 48.35 (C-18), 48.07 (C-19), 151.08 (C-20), 29.91 (C-21), 40.08 (C-22), 28.08 (C-23), 15.48 (C-24), 16.21 (C-25), 16.05 (C-26), 14.63 (C-27), 18.09 (C-28), 109.43(C-29), 19.39 (C-30).

Several studies have been conducted on species belonging to the same family (Annonaceae) where bioactive compounds have been identified. For instance *O*-methylmoschatoline and lysicamine (isolated from *Alphonsea cylindrica* barks) [23], atherospermidine (isolated from *Jatropha curcas* stem bark) [24], liriodenine (isolated from *Cananga odorata Hook*) [25], stigmasterol (isolated from *Polyalthia cauliflora var. cauliflora*) [19], and lupeol (isolated from white oak leaves) [26]. However, no studies were reported for *P. lateriflora*. As such, the present results can serve as a first reference for the identification and evaluation of the effectiveness of the phytochemicals from this species. The current study also reported for the first time the presence of the lupeol, which has never been reported in any other plant belonging to the genus *Polyalthia*.

As for the antioxidant activity (Table 1), methanol extract showed strongest activity with IC_{50} value of 128.22± 6.73 µg/ml compared to DCM and hexane extracts, and all pure compounds as revealed by their DPPH radicals scavenging activity at 500 µg/ml. Both atherospermidine and liriodenine showed weak antioxidant activity, whereas, stigmasterol was inactive. All the isolated compounds were active against MCF-7 cells with IC_{50} values of 42-89 µg/ml. *O*-methylmoschatoline possessed the highest cytotoxic effect among the alkaloids with IC_{50} value of 56 µg/ml. As for the steroid compounds, lupeol is more potent compared to stigmasterol with IC_{50} value of 73 µg/ml.

Table 1: Antioxidative and cytotoxic activities of *P. lateriflora* (Bl.) King stem bark

Extract/Compound	Antioxidant activity (% I at 500 µg/mL)	Anticancer activity (IC_{50})µg/mL
O-methylmoschatoline	ND	56
Atherospermidine	20.17±9.74; IC_{50}>500	89
Lysicamine	ND	74
Liriodenine	10.73±0.45; IC_{50}>500	86
Stigmasterol	NA	73
Lupeol	ND	42
Methanol extract	89.52±1.81; IC_{50}=128.22±6.73	NA
DCM extract	32.45±8.56; IC_{50}>500	NA
Hexane extract	NA	NA

NA: no activity (at the tested concentration range 3.9 – 500 µg/mL for antioxidant; 0-100 µg/ml for cytotoxicity); ND: not determined.

4. Conclusion

This study reported for the first time the compounds and bioactivities of *P. lateriflora* (Bl.) King stem bark. Six compounds (*O*-methylmoschatoline, atherospermidine, lysicamine, liriodenine, stigmasterol and lupeol) were identified. These findings showed that *Polyalthia* plant is very rich with an oxoaporphine type of alkaloid as well as steroid. Lupeol is reported for the first time in the genus *Polyalthia* and possessed the most potent cytotoxic effect compared to other isolated compounds.

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References

1. M G e Silva, A P de Oliveira, C de S Araújo, É M de Lavor, J C Silva, R L Mendes, C do Ó Pessoa, M P Costa, J R G da S Almeida. "Phytochemical screening, cytotoxicity and acute toxicity of *Annona vepretorum* Mart (Annonaceae) leaf extracts", *Tropical Journal of Pharmaceutical Research* **16**:597–604 (2017).
2. R M K Saunders, Y C F Su, and B Xue. "Phylogenetic affinities of *Polyalthia* species (Annonaceae) with columellar-sulcate pollen: Enlarging the Madagascan endemic genus "*Fenerivia*" *Taxon* **60**:1407–1416 (2011).
3. Z Yu, M Zhuo, X Li, Y Fu, G Chen, X Song, C Han, X Song, Q JFan. "A new norsesquiterpene from the roots of *Polyalthia laui*," *Nat. Prod. Res.* **14**:1687-1692 (2017).
4. S. A. Zaki *et al.*, "In vitro antioxidant and anti‐inflammatory potential of *Polyalthia longifolia* in rats," vol. 44, no. 2, pp. 277–279 (2012).

5. S Machana, N Weerapreeyakul, S Barusrux. "The anticancer effect of the extracts from *Polyalthia evecta* against human hepatoma cell line (HepG2)", *Asian Pac. J. Trop. Biomed*., **2(5)**:368–374 (2012).
6. M. Verma *et al.* "In vitro cytotoxic potential of *Polyalthia longifolia* on human cancer cell lines and induction of apoptosis through mitochondrial dependent pathway in HL-60 cells", *Chem. Biol. Interact*. **171**:45–56 (2008).
7. L.-J. Wu *et al.* "One new berberine from the branches and leaves of *Polyalthia obliqua* Hook.f. & Thomson", *Nat. Prod. Res.* **6419**:1–6 (2016).
8. S Kanokmedhakul, K Kanokmedhakul, R Lekphrom. "Bioactive constituents of the roots of *Polyalthia cerasoides*", *J. Nat. Prod.* **2**:1536–1538 (2007).
9. V Kumar, N Tiwari, P Gupta, S Verma, A Pal. "Phytomedicine A clerodane diterpene from *Polyalthia longifolia* as a modifying agent of the resistance of methicillin resistant *Staphylococcus aureus*", *Phytomedicine*, **23(6)**:654–661 (2016).
10. K Bhattacharya, H R Chand, J John, M V Deshpande. "Clerodane type diterpene as a novel antifungal agent from *Polyalthia longifolia var. pendula*", *Eur. J. Med. Chem.* **94**:1–7 (2015).
11. K V Katkar, A C Suthar, V S Chauhan, *P. Longifolia*. "The chemistry, pharmacologic, and therapeutic applications of Polyalthia longifolia", 4(7) (2010). Pharmacognosy Reviews.
12. K V Sashidhara *et al.* "European Journal of Medicinal Chemistry Discovery of a new class of HMG-CoA reductase inhibitor from *Polyalthia longifolia* as potential lipid lowering agent", *Eur. J. Med. Chem.* **46(10)**:5206–5211 (2011).
13. S L Jothy, Y Chen, J R Kanwar, S Sasidharan. "Evaluation of the genotoxic potential against H_2O_2 -radical-mediated DNA damage and acute oral toxicity of standardized extract of *Polyalthia longifolia* leaf", *Evidence-based Complement Altern. Med*. (2013).
14. K Annan *et al*. "Antiplasmodial constituents from the stem bark of *Polyalthia longifolia var pendula*," *Phytochem. Lett.* **11**:28–31 (2015).
15. T Kowithayakorn, M Ishibashi. "Cerasoidine, a bis-aporphine alkaloid isolated from *Polyalthia cerasoides* during screening for Signal Inhibitors", *J Nat Prod*. **79(8)**:2083-2088 (2016).
16. Z X Yu *et al.* "New clerodane diterpenoids from the roots of *Polyalthia laui*", *Fitoterapia* **111**:36–41 (2016).
17. N A Ghani, N Ahmat, N H Ismail, I Zakaria. "Flavonoid constituents from the stem bark of *Polyalthia cauliflora var cauliflora*", **5(8)**:154–158 (2011).
18. N A Ghani, N Ahmat, N H Ismail, I Zakaria, N K N a Zawawi."Chemical constituents & cytotoxic activity of *Polyalthia cauliflora var. cauliflora*", *Res. J. Med. Plant* **6(1)**:74–82 (2012).
19. S. Boonpangrak *et al.*, "Antimicrobial and cytotoxic acetogenin from Polyalthia debilis," vol. 5, no. 3, pp. 13–18, 2015.
20. A Hatamnia, N Abbaspour, R Darvishzadeh. "Antioxidant activity and phenolic profile of different parts of Bene (Pistacia atlantica subsp. kurdica) fruits", Food Chem. 145:306–311 (2014).
21. Phirdaous Abbas, Yumi Zuhanis Has-Yun Hashim, Azura Amid, Hamzah Mohd Salleh, Parveen Jamal and Irwandi Jaswir. "Anti-cancer potential of agarwood distillate. *J. Pure App Microbiol*. ", 871-874 8 (May 2014 Special Edition).
22. Vichai, V. and K. Kirtikara, "Sulforhodamine B colorimetric assay for cytotoxicity screening". *Nat Protoc*, 1(3): p. 1112-6 (2006).
23. M A Talip *et al.* "New azafluorenone derivative and antibacterial activities of *Alphonsea cylindrica* barks", *Nat. Prod. Sci.* **23(3)**:151–156 (2017).
24. D Das Gupta, M E Haque, M N Islam, S Rahman, A M Hasan, B A Shibib. "Alkaloid and steroid from the stem bark of *Jatropha curcas* (Euphorbiaceae)", *Dhaka Univ. J. Pharm. Sci.* **10(1)**:9–11 (2012).
25. K Husain, J A Jamal, J Jalil. "Phytochemical study of *Cananga odorata Hook, F. & Thoms* (Annonaceae)", *Int. J. Pharm. Pharm. Sci.* **4 suppl. 4**:465–467 (2012).
26. J A Sánchez-Burgos *et al.* "Isolation of lupeol from white oak leaves and its anti-inflammatory activity", *Ind. Crops Prod.* **77**:827–832 (2015).