INVESTIGATION OF BIOACTIVE PHYTOCONSTITUENTS AND THE BIOLOGICAL ACTIVITIES OF SOME MYANMAR TRADITIONAL MEDICINAL PLANTS

PhD (DISSERTATION)

NI NI THAN

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF YANGON
MYANMAR

DECEMBER, 2005

was isolated for the first time. The other compounds were butein (**26**), butin (**27**), monospermoside (butein-3-ß-D-glucoside) (**28**), ß-sitosterol glucoside (**30**), sulphurein (**31**), isomonospermoside (butin-3-glucoside) (**32**), isocoreopsin (Butin-7-glucoside) (**33**), butrin (**34**), isobutrin (**35**), ergost-5-en-3ß-ol (**36**), stigmasterol (**37**), ß-sitosterol (**38**), 6,10,14-trimethyl-2-pentadecanone (**39**), methyl hexadecanoate (**40**), methyl octadecanoate (**41**), heptacosane (**42**). Eleven pure compounds were isolated from the leaves of *Eclipta alba*, namely stigmasterol (**43**), wedelolactone (**44**), stigmasterol glucoside (**45**), Eclalbasaponin II (**46**), eclalbasaponin I (**47**), apigenin (**48**), luteolin (**49**), apigenin-7-O-sulphate (**50**), luteolin-7-O-sulphate (**51**), luteolin-7-O-glucoside (**52**) and indole-3-carboxylic acid (**53**). Out of the flavonoid sulphates, apigenin-7-O-sulphate, luteolin-7-O-sulphate and indole-3-carboxylic acid were isolated for the first time from the leaves of *E. alba*.

In the biological activities, primary screening was carried out for the antitumour activity of 21 compounds (compound **1-9** from *P. niruri*, compound **16-21** from *E. scaber*, compound **26**, **34** and **35** from *B. monosperma* and compound **44, 47** and **51** from *E. alba*) using six cell lines with two different concentrations.

Four compounds from *E. scaber* (deoxyelephantopin (**17**), 17,19-dihydrodeoxyelephantopin (**18**) iso-17,19-dihydrodeoxyelephantopin (**19**), lupeol (**16**),), one compound from *B. monosperma* (butein, **26**), one compound from *E. alba* (wedelolactone, **44**) which showed activity in the primary screening were further screened by 36 cell lines using five different concentrations.

The **new** substances (from *E. scaber*) 17,19-dihydrodeoxyelephantopin (**18**) and iso-17, 19-dihydrodeoxyelephantopin (**19**) exhibited a mean IC_{70} value of 4.0 µg/ml and 4.3 µg/ml respectively, compared to a mean IC_{70} value of 1.1 µg/ml for **17**. All three compounds were active against the melanoma derived cell line MEXF 394 NL. The compound **17** effected pronounced activity in the mammary cancer cell line MAXF401 NL. **18** was highly effective in the renal cancer cell line RXF 944L and **19** showed marked

activity to the large cell line lung cancer LXFL-526L. Compound **16** showed activity against central nerve system cells CNXFSF 268. Compound **26** (from *B. monosperma*) exhibited activity against the gastric cancer cell line GXF 251L, and **44** showed activity against the ovarian cancer cell line OVXF1619L. Four major compounds isolated from *E. scaber* exhibited pronounced antitumour activity, and due to their selectivity, should be used for the treatment of melanoma, mammary, renal and lung cancers. From these tests, as we are getting not only the cellular toxicity but also the selectivity, results will allow us to evaluate the potential medical value of the metabolites.

Antiviral activity of four plant extracts and the pure compounds wedelolactone (**44**) (isolated from *E. alba*), and butrin (**34**) (isolated from *B. monosperma*) was tested by using human lung endothelial cell line A-549. *E. scaber* EtOH extract showed antiviral activity at a diluation of 1:300. Wedelolactone (**44**) showed cytotoxic activity at a concentration of 100 μg/ml.

The antioxident activity of fourteen pure compounds, namely isoquercetin (**2**), gallic acid (**3**), brevifolin carboxylic acid (**4**), methyl brevifolin carboxylate (**5**), niruri flavone (**8**) and quercetin-3-O-ß-D-glucopyranosyl-(1→4)-α– rhamno-pyranoside (**9**) from *P.niruri*, butein (**26**), butin (**27**), monospermoside (butein-3-ß-D-glucoside, **28**), sulphurein (**31**), isomonospermoside (butin-3-glucoside, **32**), butrin (**34**), isobutrin (**35**), from *B.monosparma* and wedelolactone (**44**) from *E.alba* were tested by using improved an ABTS cation radical reduction assay. All the compounds were capable of reducing approximately half of the cation radical at only 10 μM. The new compound niruri flavone (**8**) reduced ABTS cation radical at 20μM and the highly active compound gallic acid (**3**) showed reduction at 2 μM.

Since a radical scavenger turns into a free radical itself after interaction with a radical, and since a reducing agent may autooxidize, it is important to test for potential prooxidant activity *in vivo*. For this purpose, bioluminescent dinoflagellate *Lingulodinium polyedrum* was monitored as an indicator of oxidative stress.

All the fourteen compounds which showed efficient scavenging capacity (2-20 μM) with ABTS cation radical were tested using concentrations of 10 and 100 μM. Gallic acid (**3**), niruriflavone (**8**), quercetin-3-O-ß-D-glucopyranosyl (1→4) α– rhamnopyranoside (**9**), butin (**27**) and wedelolactone (**44**) proved to be prooxidant in the assay. Gallic acid (**3**), which showed high scavenging capacity (at 2 μM) proved to be highly toxic. This showed that gallic acid (**3**) can scavenge free radicals forming prooxidant intermediates.

Isoquercetin (**2**), brevifolin carboxylic acid (**4**), methyl brevifolin carboxylate (**5**), and isomonospermoside (butin-3-glucoside) (**32**), which scavenged free radicals without forming prooxidant intermediates were further tested for protection of *Lingulodinium polyedrum* against toxicity by the prooxidant paraquat.

In this experiment, isomonospermoside (butin-3-glucoside, **32**) showed no sign of toxicity after microscopic inspection of the *L. polyedrum* cells, although it did not restore the full height of the glow peak maximum. It has been shown that this test on a cellular level provides much more information than simple chemical tests.

In the anti-HBsAg like activity, EtOH extracts of *P. niruri*, *E. alba*, *B. monosperma* exhibited 1/16 (32 times), 1/32 (16 times), 1/64 (8 times) test serum titre reduction, respectively. *E. scaber* EtOH extracts showed no significant activity of the three pure compounds butein (**26**), monospermoside (butein-3-ß-D-glucoside, **28**), isobutrin (**35**) isolated from *B. monosperma*, the compounds monospermoside (butein-3-ß-D-glucoside, **28**), and isobutrin (butein-3,4'-ß-D-diglucoside, **35**) showed pronounced activity by 1/8 (64 times) test serum titre reduction at a concentration of 4 mg/ml.

Key words: *Phyllanthus niruri*; *Elephantopus scaber*; *Eclipta alba*, *Butea monosperma*, antiviral activity; antitumour activity; antioxidant and prooxidant activities; ABTS cation radical; bioluminescent dinoflagellate; *Lingulodinium polyedrum*; antihepatitis B surface antigen like activity.