IN VITRO ASSESSMENT OF THE ANTI-HEPATITIS B VIRAL ACTIVITY OF SELECTED MYANMAR MEDICINAL PLANTS AND IDENTIFICATION OF ACTIVE PRINCIPLE FROM BIOASSAY GUIDED FRACTIONS

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ABSTRACT

In vitro anti-hepatitis B viral (anti-HBV) activity of selected Myanmar traditional medicinal plants, namely, *Clerodendrum neriifolium* Wall (Fam. Verbenaceae) (Pyae-sone), leaves, *Cassia fistula* Linn (Fam. Leguminosae) (Ngu), bark, and *Swertia chirata* Buch Ham (Fam. Gentianaceae) (Pan-kha), aerial parts, have been investigated by using ELISA (enzyme link immunosorbent assay) test kits. Two different concentrations (4 mg cm^{-3} and 6 mg cm^{-3} in PBS buffer) of each ethanolic crude extracts (50% and 95% ethanolic extract) were prepared for these plant samples. All ethanolic extracts of "Ngu" bark (HBsAg titre 1/128) and "Pan-kha" (HBsAg titre 1/512) showed significant anti-hepatitis B virus surface antigen (HBsAg) like activity.

Extraction, isolation, solvent partition, successive column chromato-graphic separation on silica gel and crystallization provided β-sitosterol (**I**) (0.02% yield, m.p. 138°C) from petroleum ether extract, and methyl cinnamate (**II**) (0.013% yield, m.p. 172°C) from ethylacetate extract of "Pyae-sone". Catechin (**III**) (cyanidan-3-ol) (0.0213% yield, m.p 172-174°C) has been isolated from the ethanolic extract of "Ngu" bark after column chromatographic separation on silica gel followed by sephadex LH-20 and crystallization. Decussatin (**IV**) (1-hydroxy-3,7,8-trimethoxy xanthone) (0.033% yield, m.p 151°C), isobellidifolin (**V**) (1,3,8-trihydroxy-5-methoxy xanthone) (0.167% yield, m.p 264-266°C), acacetin-6-C-glucoside (**VI**) (4'-methoxy-5,7-dihydroxy flavone 6-C glucoside) (0.05% yield, m.p 198-200°C) have been isolated from ethanolic extract of "Pan-kha". It is the first report for the presence of **VI** in "Pan-kha" plant.

All isolated constituents were identified by melting point determination and spectroscopic measurements. All of them except **I** were

screened for the presence of anti-HBsAg like activity using ELISA kit. Catechin (III) showed the most potent anti-HBsAg like activity among these isolated compounds and especially for HBsAg titre 1/16.

Present investigation revealed that either crude plants or ethanolic extracts of "Ngu" bark and "Pan-kha" could be used in the treatment of HBV infection as claimed by Myanmar traditional medicinal practitioners. Indeed, ethanolic crude extract of "Ngu" bark may be more effective than that "Pan-kha".

The anti-HBsAg like activity of III of "Ngu" bark is higher than that of ethanolic crude extract. Thus, III may be responsible for the plant to exhibit anti-HBV activity and may be of therapeutic value in treating viral hepatitis B infection. The activity of IV, V and VI of Pan-kha were lower than that of ethanolic crude extract showing the therapeutic superiority of crude ethanolic extract over single isolated constituents.