

## A219 Bioassay guided isolation of anti-HIV active compounds from the methanol extract of *Aleurites moluccana* Husks

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*Aleurites moluccana* (L.) Willd. (*Euphorbiaceae*), also known as candlenut tree, is native to Malaysia and Polynesia, and cultivated in many tropical and subtropical countries. Leaves and stem bark are traditionally used in Hawaiian folk medicine for treatment of a variety of infections and inflammations, as well as for treatment of tumors, ulcers, headache, fevers, diarrhoea and gonorrhoea.

Dried husk of *Aleurites moluccana* collected in Hawaii has been tested for its antiviral activity. Various extracts were prepared in a percolator with solvents of increasing polarity: n-hexane, dichloromethane (DCM), and methanol. Each extract was dried (under reduced pressure, 36°C) and evaluated for its *in vitro* inhibitory effect on HIV-1 (III<sub>B</sub>) and HIV-2 (ROD) replication and cytotoxicity in human lymphocyte MT-4 cells, using the MTT-based method.

The DCM and methanol extract showed the strongest activity against HIV-1 and HIV-2, the methanol extract being superior in antiviral activity as compared to the DCM extract.

The methanol extract was fractionated by column chromatography on Sephadex LH-20. Each fraction was tested for its anti-HIV activity and only the fractions with a selectivity index that exceed 10 were further examined (selectivity index (SI) value being defined as CC<sub>50</sub>/EC<sub>50</sub> or ratio of the 50% cytotoxic concentration/50% antivirally effective concentration).

The more pure the fraction, the higher the observed anti-HIV activity and selectivity. The first chemically pure active compound isolated from the methanol extract was identified as being the phytosterol,  $\beta$ -sitosterol-3- $\beta$ -D-glucopyranoside [HIV-2(ROD) in MT-4 cells, SI=1.02]. Phytosterols have been proven to have a direct immune modulatory activity on human lymphocytes. The anti-HIV target for  $\beta$ -sitosterol-3- $\beta$ -D-glucopyranoside is currently unknown.

## A220 Anti-herpes simplex virus type 2 activity of blue green algae *Spirulina platensis*

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Herpes simplex viruses (HSVs) belong to family *Herpesviridae* and are divided into 2 types; type 1 and 2. Infection from the Herpes simplex viruses have been a public health concern. Herpes simplex virus type 1 infects the oral cavity, eyes, skin area and brain, while HSV-2 infection is at genitalia. An inhibitory activity of blue green algae, *Spirulina platensis* against herpes simplex virus type 2 infections was determined by plaque reduction assay. Cytotoxicity test of algal suspension was tested on Vero cells before investigating of its antiviral activity. The results showed that the suspension of *Spirulina platensis* at a concentration of £ 0.78 mg/ml was not toxic to the cells. The inhibitions of HSV-2 infection were 81.7 % and 47.8 % comparing to control when using 0.390 and 0.195 mg/ml of algal suspension, respectively. The amount of viruses at 6, 24 and 30 hours after infection were determined after treatment the HSV-2 (1x10<sup>6</sup> PFU/ml) with algae suspension comparing to control and an antiviral agent, acyclovir. It was found that after 30 hours of HSV-2 infection, the amount of virus was decreased from 4.6 x 10<sup>6</sup> PFU/ml to 7.9 x 10<sup>5</sup> PFU/ml and < 5 PFU/ml after treatment with 0.78 mg/ml of algal suspension and 0.5 mg/ml of acyclovir, respectively. Therefore, it is possible to develop a natural product; *Spirulina platensis* as an anti-HSV drug in the future.

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**References:** 1. Hayashi, T. et al. (1996). *J. Nat. Prod.* 59, 83-87. 2. Kurokawa, M. et al. (1995). *Antiviral Res.* 27, 19-37.