

SCREENING OF ANTIVIRAL, ANTICANCER, ANTINEMATODE AND ANTIMICROBE FROM MEDICINAL PLANTS OF ORANG ASLI

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Introduction

Plants are an important source of therapeutics from which 25% of the pharmaceuticals in current use have been derived (Farnsworth and Bingel, 1977). Anticancer drugs, such as the indole alkaloids vincristine and vinblastine, and podophyllotoxin derivatives etoposide and teniposide, are prominent chemotherapeutics of plant origin which were either obtained directly through isolation or derived from lead structures. The screening efforts have resulted in the discovery of several prospective antiviral and anticancer compounds currently undergoing clinical trials of which taxol is the most notable example. Although extensive phytochemical surveys have been carried out on the flora of Malaysia, only a few reports deal with screening for pharmacological activities such as antimicrobial, antitumour, antitumour-promoting and cardiovascular-related activities (Ali et al. 1995). In the present work we screened local and introduced plant species widely used as anti-infective and anticancer agents in Malaysian indigenous medicine, comprising traditional, ethno- and folk-medicine, for antiviral, cytotoxic and antimicrobes and antinematode activities.

Materials and Methods

The antiviral test was performed according to the simplified plaque reduction assay. Microtitre plates with confluent monolayer cultures of Vero cells were filled with 100 µl of plant extract serially diluted in RPMI-1640 medium. This was followed by the addition of 100 µl of medium containing ca. 30 plaque forming units (pfu) of HSV-1 or 10 pfu of VSV, per well of confluent Vero cells. Antiviral activity was then scored using an inverted microscope (low power) as the non-cytotoxic minimum inhibitory concentration (MIC, mg/ml) which totally prevented cytopathic effects (CPE). Cytotoxicity assay was carried out according to Shier (1983) against various cancerous cell lines. Cytotoxicity was determined as the concentration of plant extract which reduced cell number by ca. 50% with reference to the control (CD₅₀, mg/ml). Antimicrobial activity of plant extracts were determined using disc diffusion assay against various target microbes (Bauer et al. 1966). For antitumour promotion activity a convenient short-term *in vitro* assay, the Epstein-Barr virus (EBV) induced by phorbol 12-myristate 13-acetate and sodium-*n*-butyrate activation was used as described by Ito et al. (1981).

Results and Discussion

The overall results of the 61 plants from 33 families screened for antiviral and cytotoxic activities showed that 28 species

(46%) were negative results for all three tests, 26 species (43%) exhibited antiviral activity and the 18 species (30%) showed cytotoxicity. Preliminary screening of the crude ethanol extracts of 19 'ulam' from 15 families for antimicrobial activity was performed qualitatively using the disc diffusion assay. Six of these 'ulam' extracts (32%), i.e. *Anacardium occidentale*, *Garcinia atroviridis*, *Averrhoa bilimbi*, *Polygonum minus*, *Diplazium esculentum* and *Elingera elatior* yielded clear inhibition zones around the discs. Of these six, more were active against Gram-negative bacteria (six extracts, 32%) than against Gram-positive bacteria or fungi, i.e. four extracts (21%) each. Three (16%) extracts were active against *Bacillus cereus*, *Escherichia coli* and *Cryptococcus neoformans*, respectively; five (26%) extracts against *Pseudomonas aeruginosa*, well noted for its extraordinary insusceptibility to most antibiotics; and two (11%) extracts against *Aspergillus ochraceous*. Ethanol extracts of different parts of 10 local traditional vegetables (ulam) (*Amaranthus gangeticus*, *Jussiaea linifolia*, *Eugenia polyantha*, *Trapa incisa*, *Trichosanthes anquina*, *Mangifera indica*, *Pachyrhizus erosus*, *Barringtonia mcarostachya*, *Carica papaya*, and *Coleus tuberosus*) were screened for *in-vitro* anti-tumour promoting activity using the inhibition test of Epstein-Barr virus (EBV) activation in Raji cells induced by phorbol 12-myristate 13-acetate and sodium-*n*-butyrate. All the extracts were found to have strong inhibition activity toward EBV-activation, except for leaves extract of *T. anquina*. The extracts were non-cytotoxic to the Raji cells except for the extracts of *A. gangeticus* (leaves), *B. macrostachya* (leaves), *E. polyantha* (young leaves), and *J. linifolia* (leaves) where the viability of the cells were decreased significantly.

Conclusions

The results of this preliminary study to a certain extent scientifically substantiate the pharmacological activities of plants used in Malaysian indigenous medicine and point out some plants with potential for further investigation. In addition, these results may also contribute towards the documentation of pharmacological profiles of Malaysian plants for conservation efforts and protection of biodiversity rights.

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