Antiviral and Cytotoxic Activities of Some Plants Used in Malaysian Indigenous Medicine

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Key words: antiviral activity, cytotoxicity, herpes simplex virus type-1, plant extracts, vesicular stomatitis virus.

Introduction

Plants are an important source of therapeutics from which 25% of the pharmaceuticals in current use have been derived (Farnsworth et al.and Bingel, 1977). However, of the estimated 250,000 species of higher plants existing throughout the world, only a fraction has been examined for pharmacological activities (Balick et al., 1990). Phytotherapeutics exhibit a wide range of pharmacological activities, which among others include anticancer and antiviral activities (Farnsworth et al. and Kaas, 1981; Hudson, 1989). 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 (Arcamone *et al.*, 1980). Therefore, the screening of higher plants for antiviral and anticancer agents has been actively pursued on an international scale especially by the U. S. National Cancer Institute (Farnsworth et al. and Kaas, 1981; Hudson, 1989). Furthermore, mammalian cell culture systems have greatly aided the routine screening of plant extracts and compounds for anticancer activity using cytotoxicity and antiviral activity, which previously relied upon time-consuming, expensive and cumbersome *in-vivo* models. These 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 (Wiernik *et al.*, 1987).

In the present work we screened 61 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 and cytotoxic activities. We adopted an ethnopharmacological approach to screening because it is more likely to yield a higher number of plants with significant biological activity than screening by random selection (Balick et al., 1990). The ethanolic extracts of these medicinal plants were tested for antiviral activities against both herpes simplex type-1 (DNA type) and vesicular stomatitis (RNA type) viruses using Vero cells, and the cytotoxicity assay was done using the HeLa (human cervical carcinoma) cell line.

Materials and Methods

Plant Extracts

Twenty mg samples of leaves from each plant (and fruits from Cerbera manghas) were sliced into small pieces (ca. 1 cm x 1 cm) and macerated in 60 ml of 80% (v/v) ethanol. After being left for one week at room temperature, the extracts were filtered using filter paper (Whatman No. 1) and then evaporated at 40°C under vacuum.

Cultivation of Cells

Vero and HeLa cell lines were obtained from the RIKEN Cell Bank, Tsukuba, Japan and cultured in RPMI-1640 medium supplemented with 5% (v/v) foetal calf serum (FCS), 100 IU/ml penicillin and 100 µg/ml streptomycin as a complete growth medium (CGM).

Virus Stocks

Herpes simplex virus type-1 (HSV-1) and vesicular stomatitis virus (VSV), which are DNA and RNA virus type respectively were obtained form the Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minnesota, USA. The antiviral test was performed according to the simplified plaque reduction assay (Abou-Karam et al. & Shier et al., 1990).

Cytotoxicity Assay

The assay used was the microtitration cytotoxicity assay (Shier et al., 1983). Using an inverted microscope (low power), cytotoxicity was determined as the concentration of plant extract, which reduced cell number by ca. 50% with reference to the control (CD_{50}, mg/ml).

Results and Discussion

The overall results of the 61 plants from 33 families screened for antiviral and cytotoxic activities. 28 species (46%) that gave negative results for all three tests. 26 species (43%) that exhibited antiviral activity and the 18 species (30%), which showed cytotoxicity. Eight species (13%), i.e. *Calotropis gigantea, Costus speciosus, Eugenia michelii, Hedyotis auricularia, Mentha arvensis, Orthosiphon aristatus, Polygonum minus* and *Ricinus communis* showed antiviral activity against both HSV-1 and VSV. The extracts of *Calotropis gigantea, Eugenia michelii* and *Ricinus communis* showed a similar MIC value of 0.01 mg/ml against both viruses but only *Eugenia michelii* demonstrated cytotoxicity (CD_{50}: 0.05 mg/ml). In the case of *Polygonum minus*, a similar MIC value against HSV-1 was obtained but lesser activity was shown against VSV (MIC: 0.02 mg/ml) and HeLa cells (CD_{50}: 0.1 mg/ml). Very strong anti HSV-1 but weak anti-VSV and cytotoxic activities were observed in the *Mentha arvensis* extract (MIC: 0.002 mg/ml & 0.1 mg/ml; and CD_{50}: 0.1 mg/ml, respectively). On the other hand, strong anti-VSV but weak anti HSV-1 activities were displayed by the extracts of *Costus specious* and *Hedyotis auricularia*, while weak antiviral activity against both viruses was observed in the extract of *Orthosiphon aristatus* (MIC: 0.1 mg/ml).

Selective antiviral activity towards only HSV-1 was seen in extracts from

seven plants (11%), viz. (in order of decreasing activity) *Alternanthera sessilis, Blumea chinensis, Freycinetia malaccensis, Leea indica, Euphorbia hirta, Eleusine indica,* and *Solanum americanum* with MIC values within the range of 0.001-0.1 mg/ml. Conversely, 11 plant (18%) extracts possessed selective antiviral activity against VSV, viz. (in order of decreasing activity) *Bertholletia excelsa, Acalypha indica, Piper sarmentosum, Oenanthe javanica, Mirabilis jalapa, Premna odorata, Cerbera manghas, Codiaeum variegatum, Plectranthus amboinicus, Centella asiatica* and *Morinda elliptica* with MIC values from 0.005 - 0.1 mg/ml. More plant extracts were active against VSV than HSV-1. The selective antiviral activity of some plant extracts against either HSV-1 or VSV implicates the involvement of different mechanisms of action exploiting the difference in nucleic acid composition of the viruses.

In the case of the anti HSV-1 species, only *Freycinetia malaccensis* showed cytotoxicity (CD_{50}: 0.1 mg/ml) whereas seven of the anti-VSV extracts showed cytotoxicity, i.e. (in order of decreasing activity) *Cerbera manghas, Morinda elliptica, Acalpyha indica, Centella asiatica, Codiaeum variegatum, Mirabilis jalapa* and *Piper sarmentosum ranging* from CD_{50} 0.001-0.1 mg/ml. Since VSV is a RNA-type virus, the concomitant anti-VSV and cytotoxic activities may involve a related mode of action, most probably via protein interaction. Co-existing antiviral and cytotoxic activities were found in the extracts of three species (5%), i.e. *Eugenia michelii, Mentha arvensis* and *Polygonum minus.*

Of the 18 plants showing cytotoxicity, only three species (*Acalypha indica, Cerbera manghas* and *Morinda elliptica*) showed significant activity below the cut-off value of 0.02 mg/ml suggested by Wall *et al.* (1987) and all three species exhibited anti-VSV activity. The strongest cytotoxic activity was shown by the fruits of *Cerbera manghas* (CD_{50}: 0.001 mg/ml). The fruits of *Cerbera manghas* always exhibited stronger cytotoxic (20 times) and anti-VSV activities (twice the activity) than its leaves. This suggests that a higher concentration of the bioactive compound(s) is present in the fruits of *Cerbera manghas* than the leaves.

Conclusions

The results of this preliminary study to a certain extent scientifically substantiate the pharmacological activities of 33 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. Inadequate record of the pharmacological activities of Malaysian plants may lead to the commercial exploitation of traditional knowledge by foreign parties without any benefit to the country as experienced by India in the case of the Neem tree and tumeric (Agarwal et al. and Narain, 1996).

Benefits from the study

Cytotoxic and antiviral assays were established in the laboratory. In the present work 61 species of 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 and cytotoxic activities.

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I·R·P·A INTENSIFICATION OF RESEARCH IN PRIORITY AREAS

Research funded by the Intensification of Research in Priority areas (IRPA) - Supported by IRPA RM-7 (1996-2000), Cycle 1996, Grant 03-02-04-0045.