Screening of promising chemotherapeutic candidates from plants extracts

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Abstract Over the course of our studies investigating anti-proliferative properties of compounds originating from plants against human gastric adenocarcinoma (MK-1), human uterine carcinoma (HeLa), murine melanoma (B16F10), and two human T cell lymphotropic virus type 1 (HTLV-1)-infected T-cell lines (MT-1 and MT-2), we have screened 582 extracted samples obtained from a variety of parts from 370 plants. A few extracts showed anti-proliferative activity against all cell lines, but upon further investigation, toxicity toward selected cell lines was recognized. After activity-guided fractionation, isolation of the active principles was achieved. Structure–activity relationship studies identified the components and functionalities responsible for the specific selectivity against each cancer cell line. The effect of polyacetylenes against MK-1 cells was more potent than against HeLa and B16F10 cells. The compound having a 3,4-dihydroxyphenethyl group also showed an anti-proliferative effect against B16F10 cells. Some 6-methoxyflavone derivatives and 8-hydroxy furano-coumarins were good inhibitors of HeLa cell growth. The 17 compounds whose EC₅₀ values were less than 1 nM did not show specific cellular selectivity. Because the cytotoxic effect of 24, 25-dihydrowithanolide D toward control cells was observed at a concentration about 100 times higher than those for the cancer cell lines, withanolide was identified as the most promising chemotherapeutic candidate in our experiments.

Keywords Cancer cell lines · Anti-proliferative activity · Activity-guided fractionation · Plant extracts · Active principles · Structure–activity relationship

Introduction

Development of anti-neoplastic drugs is the focus of numerous research programs around the world. Plants are the richest source of novel chemical compounds and in fact, many natural product-derived compounds have been identified as chemotherapeutic candidates [1]. For instance, vinca alkaloids, podophyllotoxins, taxanes, and camptothecins are four main classes of compounds that are well-known anti-neoplastic drugs originating from plants [2]. It is significant that over 60 % of the currently used anti-neoplastic drugs are derived from natural sources including plants [3].

Over the course of our studies investigating the anti-proliferative characteristics of compounds originating from plants against human gastric adenocarcinoma (MK-1), human uterine carcinoma (HeLa), murine melanoma (B16F10), and two human T cell lymphotropic virus type 1 (HTLV-1)-infected T-cell lines (MT-1 and MT-2), we have already reported many compounds active against cancer cell lines [4]. Herein, we report not only the screening results against the above cell lines but also the active principles and analysis of their structure–activity relationships.

Screening results

The 582 samples obtained from a variety of plant parts from 370 plants (302 genera, 104 families) were extracted with MeOH under reflux. The anti-proliferative effects of
the extracts against the MK-1, HeLa, B16F10, MT-1, and MT-2 cell lines were evaluated (Table 1). The extracts listed in Table 1 are classified in the Angiosperm Phylogeny Group III system. The extracts of the leaves of Annona squamosa (Annonaceae), the aerial parts and roots of Tylophora tanakae (Asclepiadaceae), and the leaves of Thuja occidentalis (Cupressaceae) showed the most potent anti-proliferative activities against all cell lines. The extracts of the leaves of Annona cherimola (Annonaceae), the fresh leaves of Tylophora ovata and T. ovata var. brownii (Asclepiadaceae), twigs of T. ovata, the roots of Saussurea lappa (Asteraceae), the seeds of Luffa acutangula (Cucurbitaceae), the leaves of Juniperus rigida (Cupressaceae), the woods of Haematoxylum brasiletto (Fabaceae), the rhizomes of Coptis japonica (Ranunculaceae), the roots of Ruta graveolens (Rutaceae), and the leaves of Cephalotaxus harringtonia (Taxaceae) showed decreasing levels of potency in the order listed. Homoharringtonine (Omacetaxine), a protein translation inhibitor for the treatment of chronic myelogenous leukemia, is found in the leaves of C. harringtonia [5]; therefore, the extract might show potent activity. There were a few extracts that had anti-proliferative activity against all cell lines and upon further investigation, toxicity toward selected cell lines was identified.

Table 2 shows a summary of the sensitivity of the plant extracts toward MK-1, HeLa, B16F10, MT-1, and MT-2 cells. The percentage of extracts that were active at concentrations of less than 100 μg/mL against the various cell lines were as follows: B16F10 (70 %), MK-1 (55 %), HeLa (39 %), MT-1 (23 %), and MT-2 (28 %). Adult T-cell leukemia/lymphoma (ATL) is a malignancy of mature peripheral T lymphocytes caused by HTLV-1. Although conventional chemotherapeutic regimens used against other malignant lymphomas have been administered to ATL patients, the therapeutic outcomes remain very poor. Therefore, these results suggest that a few plant extracts were sensitive to the T-cell lymphotropic virus type 1 (HTLV-1)-infected T cells (MT-1 and MT-2).

Active principles

Polyacetylenes (Fig. 1)

After activity-guided fractionation against MK-1, HeLa, B16F10, MT-1, and MT-2 cells, two active polyacetylenes, falcarindiol (1) and panaxydol (2), were isolated from the roots of Heracleum moellendorffii (Apiaceae) [6]. Six other polyacetylenes were isolated from the leaves of Angelica japonica (Apiaceae) [7] together with 1 and 2 after activity-guided fractionation against MK-1 cells. Among them, four compounds were furanocoumarin ethers of 1. It was evident that the effects of these compounds except for compound 3 against MK-1 cells were more potent than their effects against HeLa and B16F10 cells (Table 3). Because compound 2 showed 16 times greater activity when compared with its 8-hydroxy derivative (1), the presence of a hydroxy group at C-8 was presumed to reduce activity. The most potent compound was panaxydol (2), with an EC50 value of 1.2 μM against MK-1 cells. Bioactive panaxydol-type polyacetylenes in plant-derived foods have attracted attention as health-promoting compounds [8].

Lignans (Fig. 2)

After activity-guided fractionation against MK-1, HeLa, and B16F10 cells, seven lignans including deoxy-podophyllotoxin (9), (-)-deoxypodorphizone (10), and related compounds were isolated from the roots of Anthriscus sylvestris (Apiaceae) [9]. From the fruits of the same plant, two other lignans (14 and 15) were isolated together with 9 and 10 after activity-guided fractionation against MK-1, HeLa, and B16F10 cells [10]. Deoxypodophyllotoxin (9) showed higher activity than polyacetylenes against these cell lines. Etoposide, a clinically used chemotherapeutic agent against small-cell lung cancer, malignant lymphoma, and acute leukemia is a derivative of a podophyllotoxin isolated from Podophyllum peltatum (Berberidaceae) [11]. Of note is that the EC50 value of deoxypodophyllotoxin (9) was in the nanomolar range across all cell lines tested including MT-1 and MT-2 cells (Table 3). Topoisomerase II-inhibited DNA breakage was recognized as the mechanism of action of Etoposide. The structural features that are crucial for the anti-topoisomerase II activity of podophyllotoxin derivatives have been roughly identified as: bulky 7β-bulky substituent, trans-lactone in ring D, dioxolane ring in ring A, quasi-axial configuration of ring E, and 4'-hydroxy group [12].

Phenylethanoids (Fig. 3)

After activity-guided fractionation against B16F10 cells, two active phenylethanoids, acteoside (17) and isacteoside (18), were isolated from the leaves of Clerodendrum bungei and the bark of C. trichotomum (Lamiancaceae) [13]. Four other phenylethanoids including arenarioside (19) and leucosceptoside A (20) were isolated from the aerial parts of Lippia dulcis and L. canescens (Verbenaceae) together with some miscellaneous compounds after activity-guided fractionation against MK-1, HeLa, and B16F10 cells [14]. Furthermore, three other phenylethanoids (21–23) isolated from the leaves of Ligustrum purpurascens (Oleaceae) were also evaluated [15]. It was remarkable that the effect of phenylethanoids (17–23) against B16F10 cells was more potent than their effects against HeLa and MK-1 cells.
Table 1  Anti-proliferative activities of the plants extracts against MK-1, HeLa, B16F10, MT-1, and MT-2 cells

| Family          | Scientific name                  | Parts               | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|-----------------|----------------------------------|---------------------|------|------|--------|------|------|
| Acanthaceae     | Dicliptera japonica              | Aerial parts        | –    | –    | +      | –    | –    |
|                 | Justicia procumbens              | Whole part          | –    | +    | +      | ****| **   |
| Actinidiaceae   | Actinidia chinensis              | Fruits              | +    | +    | +++++  | –    | –    |
| Adoxaceae       | Sambucus chinensis               | Leaves              | –    | –    | –      | –    | –    |
|                 |                                  | Stems               | –    | –    | –      | –    | –    |
| Aizoaceae       | Tetragonia expansa               | Whole part          | –    | –    | +      | –    | –    |
| Amaranthaceae   | Achyranthes fauriei              | Roots               | +++  | +++  | –      | –    | –    |
|                 | Celosia argentea                 | Seeds               | –    | –    | –      | –    | –    |
|                 | Chenopodium ambrosioides         | Aerial parts        | –    | –    | –      | –    | –    |
|                 | Chenopodium ambrosioides var. anthelminticum | Leaves | +   | –    | +      | –    | –    |
|                 |                                  | Stems               | –    | –    | –      | NT   | NT   |
|                 | Chenopodium graveolens           | Aerial parts        | ++   | +    | +      | –    | **   |
|                 | Gomphrena globosa                | Whole part          | –    | –    | –      | –    | –    |
| Amaryllidaceae  | Allium sativum var. pekinense    | Bulbs               | +++  | +    | +++++  | –    | –    |
| Anacardiaceae   | Mangifera indica                 | Barks               | +++++ | +++++ | ++++++ | –    | –    |
|                 |                                  | Leaves              | +    | +    | +++++  | **   | –    |
|                 |                                  | Peels               | +    | +    | +      | **   | **   |
|                 |                                  | Pulp                | –    | –    | –      | –    | –    |
|                 |                                  | Seeds               | +++  | +    | +++++  | **   | **   |
| Annonaceae      | Annona cherimola                 | Barks               | +    | –    | +      | ****| **** |
|                 |                                  | Leaves              | +++++ | +++++ | ++++++ | **   | –    |
|                 | Annona muricata                  | Leaves              | +++  | +    | ++++++ | –    | –    |
|                 |                                  | Stems               | +++  | +    | ++++++ | **   | –    |
|                 | Annona reticulata                | Barks               | +    | –    | –      | –    | –    |
|                 |                                  | heartwoods          | +    | –    | –      | NT   | NT   |
|                 |                                  | Leaves              | +++  | +    | ++++++ | ********| *     | *     |
|                 | Annona squamosa                  | Leaves              | +++++ | +++++ | ++++++ | ****| **** |
|                 |                                  | Twigs               | +++  | +++  | +++    | **   | –    |
| Apiaceae        | Angelica acutiloba               | Fruits              | +    | –    | –      | NT   | NT   |
|                 |                                  | Leaves              | –    | –    | –      | NT   | NT   |
|                 | Angelica dahurica                | Fruits              | ++   | –    | +      | **   | **   |
|                 | Angelica decursiva               | Aerial parts        | –    | –    | –      | –    | –    |
|                 |                                  | Leaves              | ++   | –    | –      | NT   | NT   |
|                 |                                  | Fruits              | +    | –    | +      | **   | **   |
|                 | Angelica japonica                | Fruits              | ++   | –    | –      | NT   | NT   |
|                 |                                  | Leaves              | –    | –    | –      | NT   | NT   |
|                 | Angelica keiskei                 | Aerial parts        | –    | –    | –      | NT   | NT   |
|                 |                                  | Leaves              | –    | –    | –      | NT   | NT   |
|                 | Angelica kiusiana                | Leaves              | –    | –    | –      | –    | –    |
|                 | Angelica pubescens               | Roots               | +    | +    | +      | –    | –    |
|                 | Anethum graveolens               | Fruits              | –    | –    | –      | NT   | NT   |
|                 |                                  | Leaves              | –    | –    | –      | NT   | NT   |
|                 |                                  | Roots               | +++  | –    | –      | NT   | NT   |
| Family          | Scientific name          | Parts                  | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|-----------------|--------------------------|------------------------|------|------|--------|------|------|
|                | *Anthriscus cerefolium*  | Fruits                 | ++   | −    | −      | NT   | NT   |
|                | *Anthriscus sylvestris*  | Fruits                 | ++++ | ++++ | ++++   | NT   | NT   |
|                |                          | Leaves                 | ++++ | ++++ | ++++   | NT   | NT   |
|                |                          | Roots                  | ++++ | ++++ | ++++   | NT   | NT   |
|                | *Bupleurum falcatum*     | Leaves                 | −    | −    | −      | NT   | NT   |
|                | *Bupleurum rotundifolium*| Fruits                 | ++++ | ++  | +++    | NT   | NT   |
|                | *Carum carvi*            | Fruits                 | ++   | −    | +      | NT   | NT   |
|                |                          | Leaves                 | +    | −    | −      | NT   | NT   |
|                |                          | Roots                  | ++++ | −    | +      | NT   | NT   |
|                | *Centella asiatica*      | Leaves                 | ++   | −    | +      | NT   | NT   |
|                |                          | Aerial parts           | +    | −    | +++    | −    | −    |
|                | *Citrus japonica*        | Fruits                 | ++   | ++  | +++    | −    | −    |
|                | *Citrus officinalis*     | Leaves                 | −    | −    | −      | NT   | NT   |
|                |                          | Rhizomes               | −    | −    | +      | −    | −    |
|                | *Coriandrum sativum*     | Leaves                 | −    | −    | −      | −    | −    |
|                |                          | Fruits                 | ++   | −    | +      | NT   | NT   |
|                | *Cryptotaenia japonica*  | Leaves                 | ++   | −    | +      | NT   | NT   |
|                | *Foeniculum vulgare*     | Leaves                 | +    | −    | +      | NT   | NT   |
|                | *Glehnia littoralis*     | Fruits                 | ++++ | −   | +++    | NT   | NT   |
|                | *Heracleum moellendorffii*| Leaves                | +    | −    | −      | NT   | NT   |
|                |                          | Roots                  | ++++ | −    | −      | NT   | NT   |
|                | *Osmorhiza aristata*     | Aerial parts           | −    | −    | ++++   | −    | −    |
|                |                          | Roots                  | −    | −    | −      | **  | **  |
|                | *Peucedanum japonicum*   | Leaves                 | −    | −    | −      | **  | **  |
|                |                          | Stems, Root barks      | +    | +    | −      | **  | **  |
|                | *Peucedanum praeruptorum*| Roots                  | −    | −    | −      | **  | **  |
| Apocynaceae    | *Apocynum venetum*       | Whole part             | +    | −    | +      | −    | −    |
|                | *Cerbera manghas*        | Barks                  | NT   | NT   | NT     | **  | **  |
|                |                          | Leaves                 | ++++ | −    | +++    | NT   | NT   |
|                |                          | Aerial parts           | −    | −    | −      | ****| ****|
|                | *Trachelospermum jasminoides*| Leaves               | −    | −    | −      | **  | **  |
|                | *Trachelospermum liukiuense*| Aerial parts          | −    | −    | +      | −    | −    |
| Aquifoliaceae  | *Ilex cornuta*           | Fruits                 | −    | −    | +      | −    | −    |
|                |                          | Leaves                 | +    | −    | +      | **  | ****|
|                | *Ilex kudingcha*         | Leaves                 | NT   | NT   | NT     | −    | −    |
|                | *Ilex latifolia*         | Leaves                 | −    | −    | +      | −    | −    |
|                | *Ilex rotundula*         | Fruits                 | −    | −    | −      | **  | **  |
|                |                          | Leaves                 | +    | +    | +      | **  | **  |
| Araceae        | *Arisaema ringens*       | Tubers                 | +    | −    | +      | −    | −    |
|                | *Pinellia ternata*       | Tubers                 | −    | −    | −      | −    | −    |
| Araliaceae     | *Aralia cordata*         | Roots                  | −    | −    | −      | −    | −    |
|                | *Aralia elata*           | Barks                  | +    | −    | +++    | −    | −    |
|                |                          | Leaves                 | +    | −    | +      | −    | −    |
|                | *Dendropanax trifidus*   | Barks                  | −    | −    | +      | −    | −    |
|                |                          | Fruits                 | −    | −    | +      | −    | −    |
|                |                          | Leaves                 | +    | +    | +++    | −    | −    |
|                | *Eleutherococcus senticosus*| Root barks           | −    | −    | +      | **  | **  |
| Family          | Scientific name       | Parts               | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|-----------------|-----------------------|---------------------|------|------|--------|------|------|
| Araucariaceae   | Araucaria heterophylla| Leaves              | +    | +    | -      | -    | -    |
| Aristolochiaceae| Aristolochia spp.     | Roots               | +    | +    | -      | -    | -    |
|                | Asarum nipponicum     | Aerial parts        | +    | +    | -      | -    | -    |
|                | Asarum sieboldii      | Roots               | +    | +    | -      | -    | -    |
| Asclepiadaceae  | Asclepias curassavica | Leaves              | +    | +    | -      | -    | -    |
|                | Cynanchum caudatum    | Leaves              | -    | -    | -      | -    | -    |
|                | Marsdenia candurango  | Roots               | -    | -    | -      | -    | -    |
|                | Marsdenia tomentosa   | Leaves              | -    | -    | -      | -    | -    |
| Asclepiadaceae  | Metaplexis japonica  | Aerial parts        | -    | -    | -      | NT   | NT   |
| Periploca spp.  | Roots                 | -                   | -    | -    | -      | -    | -    |
| Tylophora ovata | Fresh leaves          | +                  | +    | +    | -      | -    | -    |
| Tylophora ovata| Fresh leaves          | +                  | +    | +    | -      | -    | -    |
| Tylophora ovata| Twigs                 | +                  | +    | +    | -      | -    | -    |
| Tylophora tanakae| Aerial parts       | +                  | +    | +    | -      | -    | -    |
| Asparagaceae    | Anemarrhena asphodeloides| Roots, Rhizomes    | -    | -    | -      | -    | -    |
| Dracaena draco  | Barks                | -                   | -    | -    | -      | -    | -    |
| Ophiopogon japonicus | Tubers            | -                   | -    | -    | -      | -    | -    |
| Asteraceae      | Achillea millefolium | Leaves              | -    | -    | -      | -    | -    |
|                | Stems                | -                   | -    | -    | -      | -    | -    |
| Adenocaulon himalaicum | Aerial parts     | +                   | +    | +    | -      | -    | -    |
|                | Roots                | -                   | -    | -    | -      | -    | -    |
| Adenostemma lavenia | Aerial parts         | +                   | +    | +    | -      | -    | -    |
| Arctium lappa   | Roots                | -                   | -    | -    | -      | -    | -    |
| Artemisia absinthium | Aerial parts     | +                   | -    | -    | -      | -    | -    |
|               | Leaves               | -                   | -    | -    | -      | -    | -    |
|               | Roots                | +                   | +    | +    | -      | -    | -    |
| Artemisia campestris | Aerial parts         | +                   | +    | +    | -      | -    | -    |
| Artemisia capillaris | Aerial parts         | +                   | +    | +    | -      | -    | -    |
|               | Roots                | +                   | +    | +    | -      | -    | -    |
| Artemisia ludoviciana var. mexicana| Aerial parts | +                   | +    | +    | -      | -    | -    |
| Aster spathulifolius | Leaves             | +                   | +    | +    | -      | -    | -    |
| Family | Scientific name | Parts | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|--------|-----------------|-------|------|------|--------|------|------|
|        |                 |       | +    | −    | +      | **   | **   |
| Aster  | verticillatum   | Aerial parts | +    | −    | +      | −    | −    |
| Bidens | frondosa        | Aerial parts | +    | +    | +      | −    | −    |
|        |                 | Roots, Rhizomes | ++++ | ++++ | +++    | **   | **   |
| Carthamus | tinctorius   | Flowers | −    | −    | −      | −    | −    |
| Centaurea | benecktius    | Leaves | +    | −    | +      | NT   | NT   |
| Chrysanthemum | vulgar | Aerial parts | +    | +    | +      | **   | **   |
| Cichorium | intybus       | Aerial parts | +    | −    | +      | −    | −    |
|        |                 | Roots   | +    | −    | +      | −    | −    |
| Cosmos | bipinnatus      | Seeds   | −    | −    | −      | −    | −    |
| Creasopetalum | crepidioides | Aerial parts | −    | −    | −      | −    | −    |
|        |                 | Roots, Rhizomes | −    | −    | +      | −    | −    |
| Crepidiastrum | lanceolatum | Aerial parts | −    | −    | −      | −    | −    |
| Eclipta | prostrata      | Whole part | +    | −    | +      | −    | −    |
| Eupatorium | stoechadosum | Leaves | −    | −    | −      | −    | −    |
|        |                 | Roots   | +    | +++  | +      | −    | −    |
|        |                 | Stems   | −    | −    | −      | −    | −    |
| Euryops | pectinatus     | Leaves | −    | −    | +      | −    | −    |
|        |                 | Stems   | −    | −    | −      | −    | −    |
| Helianthus | annuus     | Aerial parts | −    | −    | −      | **   | **   |
| Inula | helenium       | Roots   | +++  | +++  | +++    | NT   | NT   |
| Ligularia | japonica     | Leaves | −    | −    | −      | **   | −    |
|        |                 | Roots   | −    | −    | −      | −    | −    |
| Neurolaena | lobata        | Leaves | −    | +    | +      | NT   | NT   |
| Parasenecio | tebakoensis | Aerial parts | +    | −    | +      | −    | −    |
| Santolina | chamaeyparissus | Leaves | −    | −    | +      | −    | −    |
|        |                 | Stems   | −    | −    | +++    | −    | −    |
| Suaevia | lappa          | Roots   | ++++ | ++++ | ++++   | **   | ****|
| Senecio | vulgaris       | Whole part | −    | −    | −      | −    | −    |
| Siegesbeckia | glabrescens | Leaves | +    | +    | −      | −    | −    |
|        |                 | Roots   | −    | −    | −      | −    | −    |
| Sonchus | asper          | Aerial parts | −    | −    | +      | −    | −    |
| Tagetes | patula         | Aerial parts | −    | −    | +      | −    | **   |
|        |                 | Roots   | −    | −    | +      | −    | −    |
| Tridax | procumbens     | Leaves | −    | −    | −      | NT   | NT   |
| Tussilago | farfara     | Roots   | +    | −    | +      | −    | −    |
| Wedelia | prostrata     | Whole part | −    | −    | +      | **   | **   |
| Xanthium | strumarium    | Fruits  | −    | −    | −      | −    | −    |
| Balsaminaceae | Impatiens textori | Aerial parts | −    | −    | +      | **   | **   |
| Berberidaceae | Berberis japonica | Leaves | +    | ++    | +++    | −    | **   |
|        |                 | Roots   | +    | +    | ++++   | **   | **   |
| Epimedium | grandiflorum subsp. | Roots, Rhizomes | −    | −    | −      | −    | −    |
| Epimedium | sagittatum    | Aerial parts | ++++ | ++++ | ++++   | −    | −    |
| Nandina | domestica     | Barks   | +    | +    | +++    | −    | **   |
|        |                 | Leaves  | ++    | −    | +++    | −    | −    |
| Bignoniaceae | Pseudocalyymna alliaceum | Aerial parts | −    | −    | +      | **   | −    |
| Boraginaceae | Lithospermum officinale var. erythrorhizon | Roots | −    | −    | −      | −    | −    |
| Brassicaceae | Isatis indigotica | Fruits | −    | −    | +      | **   | **   |
Table 1 continued

| Family       | Scientific name          | Parts      | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|--------------|--------------------------|------------|------|------|--------|------|------|
| Lepidium apetalum | Seeds                    | ++ ++      | **   | **   |        |      |      |
| Lepidium virginicum | Whole part              | – –      | +    | –    |        |      |      |
| Thalipis arvense | Seeds                    | – –      | –    | –    |        |      |      |
| Burseraceae  | Bursara simaruba         | Fruits     | + +  | +    | –      |      |      |
| Campanulaceae | Codonopsis spp.          | Roots      | – –  | –    | –      |      |      |
| Cannabaceae  | Humulus japonicus        | Aerial parts | – – | +    | **   | **   |      |
| Caprifoliaceae | Lonicera japonica       | Flowers    | – –  | –    | –      |      |      |
| Caricaceae   | Carica papaya            | Barks      | – –  | –    | –      |      |      |
| Caryophyllaceae | Agrostrum githago     | Seeds      | +    | –    | +      | –    | –    |
| Vaccaria segetalis | Seeds              | + –      | –    | –    | –      |      |      |
| Celastraceae | Celastrus orbicularis    | Vines      | – –  | –    | –      | –    | –    |
| Euonymus alatus | Barks                   | – –      | +++  | +    | ++     | **   | **   |
| Euonymus japonicus | Barks               | – –      | –    | –    | +      | –    | –    |
| Myrtens diversifolia | Leaves             | – –      | –    | –    | +      | –    | –    |
| Chloranthaceae | Sarcandra glabra       | Roots      | –    | +++  | +      | –    | –    |
| Clusiaceae   | Garcinia subelliptica    | Barks      | +    | +    | +++++  | –    | –    |
| Garcinia xanthochymes | Leaves            | – –      | –    | –    | –      |      |      |
| Cornus officinalis | Fruits             | – –      | –    | –    | –      |      |      |
| Cumbretaceae | Terminalia chebula       | Fruits     | +    | +++  | +++++  | **   | **   |
| Commelinaceae | Commelina communis      | Whole part | – –  | –    | +      | –    | –    |
| Cornaceae    | Camptotheca acuminata    | Fruits     | ++++  | ++++ | +++    | **   | **   |
| Cornus officinalis | Fruits             | – –      | –    | –    | –      |      |      |
| Crasulaceae  | Bryophyllum pinnatum     | Aerial parts | +    | –    | +      | –    | –    |
| Hylotelephium erythrosticum | Roots     | +        | +    | ++    | –      | –    | –    |
| Orostachys japonicus | Whole part  | – –      | –    | –    | +      | –    | –    |
| Sedum azozno var. floribundum | Roots | +++     | +    | +++    | –      | –    | –    |
| Sedum tomentosum | Whole part  | – –      | –    | –    | +      | –    | –    |
| Cucurbitaceae | Actinostemma lobatum     | Aerial parts | +    | +    | –      | –    | –    |
| Citrullus colocynthis | Seeds             | – –      | –    | –    | –      |      |      |
| Gynostemma pentaphyllum | Aerial parts | – –      | –    | –    | –      |      |      |
| Lagenaria leucantha var. gourda | Fruits | ++++   | ++++ | +      | ****  | **   |      |
| Maytenus diversifolia | Leaves          | – –      | –    | –    | +      | –    | –    |
| Chloranthaceae | Sarcandra glabra       | Roots      | –    | +++  | +      | –    | –    |
| Caricaceae   | Carica papaya            | Barks      | – –  | –    | –      | –    | –    |
| Caryophyllaceae | Agrostrum githago     | Seeds      | +    | –    | +      | –    | –    |
| Vaccaria segetalis | Seeds              | + –      | –    | –    | –      |      |      |
| Celastraceae | Celastrus orbicularis    | Vines      | – –  | –    | –      | –    | –    |
| Euonymus alatus | Barks                   | – –      |+++  | +    | ++     | **   | **   |
| Euonymus japonicus | Barks               | – –      | –    | –    | +      | –    | –    |
| Maytenus diversifolia | Leaves             | – –      | –    | –    | +      | –    | –    |
| Chloranthaceae | Sarcandra glabra       | Roots      | –    | +++  | +      | –    | –    |
| Clusiaceae   | Garcinia subelliptica    | Barks      | +    | +    | +++++  | –    | –    |
| Garcinia xanthochymes | Leaves            | – –      | –    | –    | –      |      |      |
| Pulp         | + –                      | –    | –    | –    | –      |      |      |
| Seeds        | + –                      | –    | –    | –    | –      |      |      |
| Stems        | + –                      | –    | –    | –    | –      |      |      |
| Heartwoods   | – –                      | –    | –    | –    | –      |      |      |
| Leaves       | + –                      | –    | –    | –    | –      |      |      |
| Barks        | + –                      | –    | –    | –    | –      |      |      |
| Fruits       | + –                      | –    | –    | –    | –      |      |      |
| Aerial parts | + –                      | –    | –    | –    | –      |      |      |
| Roots        | + –                      | –    | –    | –    | –      |      |      |
| Seeds        | + –                      | –    | –    | –    | –      |      |      |
| Stems        | + –                      | –    | –    | –    | –      |      |      |
| Lagenaria leucantha var. gourda | Fruits | ++++   | ++++ | +      | ****  | **   |      |
| Maytenus diversifolia | Leaves          | – –      | –    | –    | +      | –    | –    |
| Chloranthaceae | Sarcandra glabra       | Roots      | –    | +++  | +      | –    | –    |
| Caricaceae   | Carica papaya            | Barks      | – –  | –    | –      | –    | –    |
| Caryophyllaceae | Agrostrum githago     | Seeds      | +    | –    | +      | –    | –    |
| Vaccaria segetalis | Seeds              | + –      | –    | –    | –      |      |      |
| Celastraceae | Celastrus orbicularis    | Vines      | – –  | –    | –      | –    | –    |
| Euonymus alatus | Barks                   | – –      |+++  | +    | ++     | **   | **   |
| Euonymus japonicus | Barks               | – –      | –    | –    | +      | –    | –    |
| Maytenus diversifolia | Leaves             | – –      | –    | –    | +      | –    | –    |
| Chloranthaceae | Sarcandra glabra       | Roots      | –    | +++  | +      | –    | –    |
| Clusiaceae   | Garcinia subelliptica    | Barks      | +    | +    | +++++  | –    | –    |
| Garcinia xanthochymes | Leaves            | – –      | –    | –    | –      |      |      |
| Pulp         | + –                      | –    | –    | –    | –      |      |      |
| Seeds        | + –                      | –    | –    | –    | –      |      |      |
| Stems        | + –                      | –    | –    | –    | –      |      |      |
| Heartwoods   | – –                      | –    | –    | –    | –      |      |      |
| Leaves       | + –                      | –    | –    | –    | –      |      |      |
| Barks        | + –                      | –    | –    | –    | –      |      |      |
| Fruits       | + –                      | –    | –    | –    | –      |      |      |
| Aerial parts | + –                      | –    | –    | –    | –      |      |      |
| Roots        | + –                      | –    | –    | –    | –      |      |      |
| Seeds        | + –                      | –    | –    | –    | –      |      |      |
| Stems        | + –                      | –    | –    | –    | –      |      |      |
| Lagenaria leucantha var. gourda | Fruits | ++++   | ++++ | +      | ****  | **   |      |
| Maytenus diversifolia | Leaves          | – –      | –    | –    | +      | –    | –    |
| Family        | Scientific name                              | Parts       | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|--------------|---------------------------------------------|-------------|------|------|--------|------|------|
| Lagenaria    | *leucantha* var. *microcarpa*                | Fruits      | ++++ | ++++ | +++    | NT   | NT   |
|              |                                             | Seeds       | ++++ | ++++ | +++    | –    | –    |
| Luffa        | *acutangula*                                | Aerial parts| +   | +   | +      | **   | **   |
|              |                                             | Seeds       | ++  | ++++ | ++++   | **   | **** |
| Luffa        | *aegyptica*                                 | Fruits      | –   | –   | –      | –    | –    |
| Momordica    | *charantia*                                 | Aerial parts| –   | –   | –      | –    | –    |
|              |                                             | Fruits      | –   | –   | –      | NT   | NT   |
|              |                                             | Seeds       | –   | –   | –      | –    | –    |
| Momordica    | *cochininchensis*                           | Fruits      | –   | –   | –      | NT   | NT   |
| Sicana       | *odorifera*                                 | Fruits      | +   | +   | –      | NT   | NT   |
| Trichosanthes| *kirilowii* var. *japonica*                 | Roots       | +++ | +++ | +      | –    | –    |
| Biota        | *orientalis*                                | Leaves      | ++  | ++  | +++    | **   | **   |
| Juniperus    | *chinensis* var. *kaizuka* Hort.            | Leaves      | +   | +   | +      | **   | **   |
|              |                                             | Stems       | +   | +   | +      | –    | –    |
| Juniperus    | *rigida*                                    | Leaves      | +++ | +++ | ++++   | **** | **** |
|              |                                             | Stems       | +   | +++ | +      | **   | **   |
| Thuja        | *occidentalis*                              | Leaves      | ++++| ++++| ++++   | *****| *****|
|              |                                             | Stems       | +   | +++ | +++    | *    | **   |
| Cupressaceae |                                             |             |     |     |        |      |      |
|               | *Cycas revoluta*                            | Leaves      | –   | –   | +      | –    | –    |
|               |                                             | Peels       | –   | –   | +      | –    | –    |
|               |                                             | Seed kernels| –   | –   | –      | –    | –    |
| Daphniphyllaceae | *Daphniphyllum macropodum*                  | Barks       | –   | +   | –      | –    | –    |
|               |                                             | Leaves      | –   | –   | +      | –    | –    |
| Elaeocarpaceae| *Elaeocarpus sylvestris* var. *ellipticus*  | Barks       | +++ | +++ | +++    | **   | **   |
|               |                                             | Leaves      | +++ | +++ | +      | **   | **   |
| Eucommiaceae | *Eucommia ulmoides*                         | Barks       | –   | –   | –      | **   | **   |
| Euphorbiaceae| *Acalypa australis*                         | Roots       | ++  | +   | +      | –    | –    |
|              |                                             | Leaves      | +   | –   | +      | –    | –    |
|              |                                             | Aerial parts| +   | +   | +      | –    | –    |
|              |                                             | Roots       | +   | +   | +      | –    | –    |
|              |                                             | Whole part  | +++ | +   | +      | –    | –    |
|              |                                             | Aerial parts| +++ | +   | +      | –    | –    |
|              |                                             | Seeds       | +++ | –   | –      | –    | –    |
| Fabaceae     | *Acacia melanoxylon*                        | Barks       | +++ | +   | +++    | –    | –    |
|              |                                             | Leaves      | +   | +   | +      | –    | **   |
|              |                                             | Flowers     | NT  | NT  | NT     | –    | –    |
|              |                                             | Roots       | –   | +   | –      | –    | –    |
|              |                                             | Seeds       | –   | –   | +      | **   | **   |
| Cassia       | *obtusifolia*                               | Seeds       | –   | –   | +      | –    | –    |
|              |                                             | Leaves      | +   | –   | +      | –    | –    |
|              |                                             | Stems       | –   | –   | +      | –    | –    |
| Erythrina    | *variegata* var. *orientalis*               | Barks       | –   | +   | –      | –    | –    |
|              |                                             | Roots       | –   | +   | –      | –    | –    |
|              |                                             | Seeds       | +   | +   | +++    | –    | –    |
|              |                                             | Leaves      | +   | –   | ++     | –    | –    |
|              |                                             | Underground parts| – | + | – | – | – |
| Family        | Scientific name                                      | Parts    | MK-1  | HeLa | B16F10 | MT-1 | MT-2 |
|--------------|-----------------------------------------------------|----------|-------|------|--------|------|------|
|              | **Glycyrrhiza uralensis**                           | Roots    | -     | +    | -      | -    | -    |
|              | **Haematoxyllum brasiliense**                       | Woods    | ++++  | ++++ | ++++   | ***  | **** |
|              | **Lonchocarpus oxacensis**                          | Roots    | +     | -    | -      | NT   | NT   |
|              | **Lonchocarpus unifoliolatus**                      | Roots    | +     | -    | -      | NT   | NT   |
|              | **Medicago polymorpha**                             | Whole part| -    | -    | -      | -    | -    |
|              | **Mellotus officinalis**                            | Whole part| -    | +    | -      | -    | -    |
|              | **Psoralea corylifolia**                            | Seeds    | +++   | +++  | ++++   | **   | -    |
|              | **Rhynchosia volabilis**                            | Seeds    | ++++  | +++  | ++++   | **   | -    |
|              | **Sophora japonica**                                | Fruits   | -     | -    | +      | **   | **   |
|              | **Trifolium dubium**                                | Aerial parts| - | +    | -      | -    | -    |
|              | **Zornia spp.**                                     | Leaves   | +     | -    | +      | -    | **   |
| Gelsemiaceae | **Gelsemium sempervirens**                          | Leaves   | -     | -    | +++    | -    | -    |
|              |                                                     | Stems    | -     | -    | +      | -    | -    |
| Geraniaceae  | **Pelargonium graveolens**                          | Leaves   | ++++  | +    | ++++   | -    | **   |
|              |                                                     | Stems    | ++++  | +    | ++++   | -    | **   |
| Iridaceae    | **Crocosmia aurea**                                 | Bulbs    | -     | -    | -      | -    | -    |
| Juglandaceae | **Juglans mandshurica var. sachalinensis**          | Barks    | ++    | ++   | +++    | -    | -    |
| Lamiaeae     | **Ajuga decumbens**                                 | Whole part| -    | -    | -      | -    | -    |
|              | **Ajuga reptans**                                   | Leaves   | -     | -    | -      | -    | -    |
|              | **Caryopteris incana**                              | Aerial parts| - | -    | +      | -    | -    |
|              | **Clerodendron thomsoniae**                         | Leaves   | -     | -    | -      | NT   | NT   |
|              | **Clerodendrum bungei**                             | Flowers  | -     | -    | -      | -    | -    |
|              |                                                     | Leaves   | -     | -    | ++     | -    | -    |
|              |                                                     | Stems    | -     | -    | -      | -    | -    |
|              | **Clerodendrum trichotomum**                        | Barks    | +     | -    | +++    | -    | -    |
|              |                                                     | Flowers  | -     | -    | -      | -    | -    |
|              |                                                     | Fruits   | -     | -    | +      | -    | -    |
|              |                                                     | Leaves   | -     | -    | +++    | -    | -    |
|              | **Elsholtzia ciliata**                              | Aerial parts| + | -    | +      | -    | -    |
|              | **Glechoma longituba**                              | Whole part| -    | -    | -      | -    | -    |
|              | **Isodon japonicus**                                | Leaves   | +     | +    | ++++   | -    | -    |
|              |                                                     | Roots    | +     | +    | -      | -    | -    |
|              |                                                     | Stems    | +     | +    | ++++   | -    | -    |
|              | **Lamium amplexicaule**                             | Whole part| -    | -    | -      | -    | -    |
|              | **Leonurus sibiricus**                              | Aerial parts| - | -    | **    | **   | **   |
|              |                                                     | Roots    | -     | -    | +      | -    | -    |
|              |                                                     | Seeds    | -     | -    | +      | -    | -    |
|              | **Rosmarinus officinalis**                          | Leaves   | ++++  | +    | ++++   | **   | **   |
|              | **Salvia miltiorrhiza**                             | Roots    | +     | -    | +      | -    | -    |
|              | **Scutellaria baicalensis**                         | Roots    | +     | +    | -      | -    | -    |
|              | **Scutellaria barbata**                             | Whole part| -    | -    | +      | **   | **   |
|              | **Teucrium japonicum**                              | Leaves   | -     | -    | +++    | -    | -    |
|              | **Vitex trifolia**                                  | Branches | NT    | NT   | NT     | NT   | -    |
| Lamiaceae    | **Cinnamomum cassia**                               | Barks    | +     | +    | +      | **   | **   |
|              |                                                     | Leaves   | -     | -    | -      | -    | -    |
|              | **Lindera strychnifolia**                           | Roots    | +     | +    | -      | -    | -    |
|              | **Persea americana**                                | Leaves   | ++++  | ++++ | ++++   | -    | -    |
|              |                                                     | Pulp     | +     | +++  | +      | -    | -    |
|              |                                                     | Seeds    | +     | +    | -      | -    | -    |
| Family         | Scientific name                          | Parts          | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|---------------|------------------------------------------|----------------|------|------|--------|------|------|
| Liliaceae     | *Fritillaria verticillata* var. *thungergii* | Twigs          | +    | +    | +      | −    | −    |
| Lythraceae    | *Capsia hyssopifolia*                    | Aerial parts   | +    | +    | +      | −    | −    |
|               |                                          | Roots          | ++   | +    | +      | −    | −    |
|               | *Punica granatola*                       | Peels          | ++++ | +    | +++    | **   | **   |
| Magnoliaceae  | *Magnolia ova*                           | Barks          | +    | +    | +      | −    | −    |
| Malvaceae     | *Abelmoschus manihot*                    | Leaves         | −    | +    | +      | −    | −    |
|               | *Althaeae cannabina*                     | Leaves         | −    | −    | −      | −    | −    |
|               | *Chorisia speciosa*                      | Immatured fruits | −  | −    | −      | −    | −    |
|               | *Corchoropsis tomentosa*                 | Fruits         | −    | −    | +      | **   | **   |
|               |                                          | Leaves         | +    | +    | +++    | −    | −    |
|               |                                          | Stems          | −    | −    | +      | −    | −    |
|               | *Gossypium arboresum*                    | Leaves         | +    | +    | ++     | −    | −    |
|               |                                          | Roots          | +    | +    | ++     | −    | −    |
|               |                                          | Stems          | +    | −    | +      | −    | −    |
|               | *Gossypium brasiliensis*                 | Leaves         | ++   | ++   | ++     | −    | −    |
|               |                                          | Roots          | −    | +    | +      | −    | −    |
|               |                                          | Stems          | −    | +    | +      | −    | −    |
|               | *Malvaviscus arboresus*                  | Leaves         | +    | −    | −      | −    | −    |
|               |                                           | Barks          | +    | +    | +      | **   | **   |
|               |                                           | Leaves         | +    | +    | +      | −    | −    |
|               |                                           | Barks          | −    | −    | −      | −    | −    |
|               |                                           | Heartwoods     | −    | −    | −      | −    | −    |
|               |                                           | Leaves         | +++  | +    | +++    | **   | **   |
| Menispermaceae| *Melia azedarach* var. toosendan*         | Fruits         | −    | −    | ++++   | **   | **   |
| Moraceae      | *Cocos trilobus*                         | Fruits         | −    | −    | +      | −    | −    |
|               |                                           | Leaves         | +    | +    | +      | −    | −    |
|               |                                           | Vines          | −    | −    | +      | −    | −    |
|               | *Stephania tetrandra*                    | Roots          | −    | −    | +      | **   | **   |
|               |                                           | Stems          | −    | −    | −      | −    | −    |
|               | *Tinospora tuberculata*                  | Stems          | −    | −    | −      | −    | −    |
| Myrtaceae     | *Ficus carica*                           | Leaves         | −    | −    | +      | −    | −    |
|               | *Ficus pumila*                           | Fruits         | +    | −    | +      | −    | −    |
|               |                                           | Leaves         | +    | +    | +++    | −    | −    |
|               |                                           | Stems          | −    | −    | +      | −    | −    |
|               | *Morus alba*                             | Root barks     | −    | −    | −      | −    | −    |
|               | *Myristica fragrans*                     | MeOH−oil       | ++   | ++   | ++     | NT   | NT   |
|               |                                           | MeOH−ppt       | −    | −    | +      | −    | −    |
| Muntingiaceae | *Muntingia calabura*                     | Fruits         | +    | −    | +      | NT   | NT   |
|               |                                           | Leaves         | +++  | +    | ++     | −    | −    |
|               | *Myrtaceae*                              | Fruits         | −    | −    | −      | −    | −    |
|               |                                           | Leaves         | +    | +    | +++    | −    | −    |
|               |                                           | Twigs          | +++  | +    | +++    | −    | −    |
|               | *Psidium cattleyanum*                    | Branches       | +++  | +    | +      | −    | −    |
|               |                                           | Fruits         | −    | −    | −      | −    | −    |
|               |                                           | Leaves         | +    | +    | +      | −    | −    |
|               | *Psidium guajava*                        | Branches       | +    | +    | +++    | −    | −    |
|               |                                           | Leaves         | +    | +    | +      | −    | −    |
|               |                                           | Twigs          | +++  | +++  | +++    | −    | −    |

Table 1 continued
| Family                | Scientific name                  | Parts            | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|----------------------|----------------------------------|------------------|------|------|--------|------|------|
| Nyctaginaceae        | Mirabilis jalapa                 | Leaves           | -    | -    | +      | -    | **   |
|                      |                                  | Roots            | +    | +    | +      | -    | -    |
| Oleaceae             | Ligustrum japonicum              | Immatured fruits | +    | -    | +      | -    | -    |
|                      |                                  | Leaves           | +    | +    | +      | NT   | NT   |
|                      | Ligustrum lucidum                | Fruits           | +    | -    | +      | -    | -    |
|                      |                                  | Leaves           | +    | -    | ++     | -    | -    |
|                      | Ligustrum ovalifolium            | Leaves           | -    | -    | +      | -    | -    |
|                      | Ligustrum purpurascens           | Leaves           | +    | +    | +      | -    | -    |
| Orchidaceae          | Dendrobium spp.                  | Aerial parts     | +    | +    | +      | -    | -    |
| Orobancheaceae       | Cistanche deserticola           | Stems            | -    | -    | +      | -    | -    |
| Oxalidaceae          | Averrhoa carambola               | Barks            | -    | -    | +      | -    | -    |
|                      |                                  | Leaves           | +    | -    | +      | -    | -    |
| Paonaceae            | Paephina lactiflora              | Roots            | -    | -    | -      | -    | -    |
| Papaveraceae         | Corydalis heterocarpa var. japonica | Aerial parts   | -    | -    | +      | **   | **   |
|                      |                                  | Roots            | +    | -    | -      | -    | -    |
|                      | Corydalis tartschaniowii var. yuhanusuo | Tubers        | +    | +    | +      | -    | **   |
| Phyllanthaceae       | Phyllanthus aciduse              | Leafstalks, Twigs | -    | -    | -      | -    | -    |
| Phyllanthus urinaria |                                  | Leaves           | +    | -    | -      | -    | -    |
| Phytolaccaceae       | Petiveria alliacea               | Leaves           | +    | +    | +++    | NT   | NT   |
| Phytoleca americana  |                                  | Roots            | -    | -    | -      | -    | -    |
| Rivina humilis       |                                  | Aerial parts     | -    | -    | +      | -    | -    |
| Piperaceae           | Piper spp.                       | Leaves           | +    | -    | +      | **   | **   |
| Pittosporaceae       | Pittosporum tobira               | Barks            | -    | -    | -      | -    | -    |
|                      |                                  | Fruits           | +    | +    | +      | -    | -    |
|                      |                                  | Leaves           | +    | -    | +      | -    | -    |
|                      |                                  | Peels            | +    | +    | +      | **   | **   |
| Plantaginaceae       | Pentstemon gloxinioides          | Leaves           | -    | -    | +      | -    | -    |
|                      |                                  | Rhizomes         | -    | -    | +++    | -    | -    |
|                      |                                  | Stems            | -    | -    | +      | -    | -    |
| Picrorhiza scrophulariiflora |                                | Rhizomes         | -    | -    | +      | -    | -    |
| Polygala tenerifolia |                                  | Roots            | -    | -    | -      | -    | -    |
| Polygala orientale   |                                  | Seeds            | +    | +    | +++    | -    | -    |
| Polygala tinctorium  |                                  | Whole part       | +    | +    | +      | -    | -    |
| Plumbaginaceae       | Plumbago capensis                | Whole part       | +    | +    | +      | -    | -    |
| Podocarpaceae        | Podocarpus macrophyllus         | Leaves           | +    | +    | +++    | -    | -    |
|                      |                                  | Stems            | +    | +    | +++    | -    | -    |
| Polygalaceae         | Polygala tenerifolia             | Roots            | -    | -    | -      | -    | -    |
| Polygonaceae         | Fallopia japonica                | Roots            | +    | +    | +      | -    | -    |
|                      |                                  | Seeds            | +    | +    | +++    | -    | -    |
|                      | Polygonum tinctorium             | Whole part       | -    | -    | -      | **   | **   |
| Rheum palmatum       |                                  | Rhizomes         | ++++ | +    | ++++   | -    | -    |
| Rumex acetosa        |                                  | Roots, Rhizomes  | -    | -    | +      | **   | **   |
| Rumex japonicus      |                                  | Roots, Rhizomes  | -    | -    | ++     | -    | **   |
| Polybiaceae          | Drymaria fortunii               | Rhizomes         | -    | -    | +      | -    | -    |
| Polybiaceae          | Phlebodium aureum               | Dried roots      | +    | -    | +      | -    | -    |
|                      |                                  | Fresh roots      | +    | -    | -      | **   | **   |
|                      |                                  | Whole part       | +    | -    | +      | -    | -    |
| Portulacaceae        | Portulaca oleracea               | Whole part       | +    | -    | +      | -    | **   |
| Family           | Scientific name | Parts               | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|------------------|-----------------|---------------------|------|------|--------|------|------|
| Primulaceae      | Ardisia crenata | Leaves              | –    | –    | +++++  | –    | –    |
|                  |                 | Roots               | +++++ | +    | +++    | **** | **** |
|                  |                 | Stems               | –    | –    | +++    | –    | –    |
| Ardisia japonica |                 | Leaves              | +    | +    | +      | –    | –    |
|                  |                 | Stems, Underground parts | +    | +    | +++    | –    | –    |
| Lysimachia japonica |             | Whole part         | –    | –    | +      | –    | –    |
| Proteaceae       | Macadamia ternifolia | Leaves            | +    | +    | +++    | –    | –    |
|                  |                 | Twigs               | +++  | ++   | +++    | NT   | NT   |
| Pteridaceae      | Pteris multifida | Aerial parts       | –    | –    | +      | –    | –    |
|                  |                 | Roots, Rhizomes     | +    | +    | +      | –    | –    |
| Ranunculaceae    | Cimicifuga simplex var. ramosa | Aerial parts  | –    | –    | +      | –    | –    |
|                  |                 | Underground parts   | –    | +    | –      | –    | –    |
|                  | Clematis paniculata | Aerial parts   | +    | –    | +      | –    | –    |
|                  | Coptis japonica | Rhizomes            | ++++ | ++++ | ++++++ | **  | **   |
|                  | Thalictrum thunbergii | Aerial parts    | –    | –    | +      | –    | –    |
|                  |                 | Underground parts   | –    | –    | +++    | –    | –    |
| Rhamnaceae       | Berchemia racemosa | Leaves            | –    | –    | +      | –    | –    |
|                  |                 | Stems               | –    | –    | +      | –    | –    |
|                  | Boehmeria dulcis | Fruits              | –    | –    | +      | –    | –    |
|                  | Zizyphus jujuba var. jujuba | Fruits  | –    | –    | –      | –    | –    |
|                  | Zizyphus jujuba var. spinosa | Seeds    | –    | –    | +      | –    | –    |
| Rehmanniaceae    | Rehmannia glutinosa var. purpurea | Roots      | –    | –    | –      | –    | –    |
| Rosaceae         | Agrimonia pilosa | Whole part         | –    | –    | ++     | –    | –    |
|                  | Chaenomeles sinensis | Fruits        | –    | –    | +      | ++   | –    |
|                  | Crataegus cuneata | Fruits             | –    | +    | –      | –    | –    |
|                  | Eryobotrya japonica | Barks          | +    | +    | +      | –    | –    |
|                  | Geum japonicum  | Aerial parts       | +    | +    | +      | –    | –    |
|                  |                 | Roots               | +    | +    | +++    | –    | –    |
|                  | Potentilla chrysanthi | Whole part    | –    | –    | +      | –    | –    |
|                  | Potentilla fragarioides var. major | Aerial parts | –    | –    | +      | –    | –    |
|                  |                 | Underground parts   | +    | +    | +++    | **  | **   |
|                  | Potentilla indica | Whole part         | +    | –    | +      | –    | –    |
|                  | Prunus armeniaca | Seeds kernels      | –    | +    | –      | –    | –    |
|                  | Rosa multiflora | Fruits              | –    | +    | –      | –    | –    |
|                  | Rubus hirsutus   | Aerial parts       | –    | –    | +      | –    | –    |
|                  | Sanguisorba officinalis var. carnea | Roots      | +    | –    | +      | –    | –    |
| Rubiaceae        | Damnocanthus macrophyllus var. macrophyllus | Leaves    | –    | –    | ++     | –    | –    |
|                  |                 | Roots               | –    | –    | –      | –    | –    |
|                  |                 | Stems               | –    | –    | –      | –    | –    |
|                  | Galium pogsnoanthum | Aerial part  | –    | –    | +      | –    | –    |
|                  | Hamelia patens  | Leaves, Twigs       | +    | –    | ++     | –    | –    |
|                  | Hedyotis diffusa | Whole part          | –    | –    | –      | –    | –    |
|                  | Paederia scandens | Fruits             | –    | +    | –      | –    | –    |
|                  |                 | Leaves              | –    | –    | –      | –    | –    |
|                  |                 | Stems               | –    | –    | –      | –    | –    |
|                  | Rubia argyi     | Roots               | +++++ | ++++ | ++++   | **  | **   |
|                  | Uncaria rhynchophylla | Hooks       | –    | –    | ++     | –    | –    |
| Rutaceae         | Boeninghausenia japonica | Aerial parts | +    | +    | +      | **  | **   |
|                  |                 | Roots               | +++  | ++++ | ++++   | **  | **   |
| Family            | Scientific name         | Parts        | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|-------------------|-------------------------|--------------|------|------|--------|------|------|
| Citrus grandis    | Peels                   | --           | --   | +    | --     | --   | --   |
| Citrus natsudaidai| Immatured fruits        | --           | --   | +    | --     | --   | --   |
| Evodia rutaecarpa | Barks                   | --           | --   | +    | --     | --   | --   |
| Orissa japonica  | Leaves                  | +            | +    | +    | --     | --   | --   |
| Phellodendron amurense | Barks             | +            | +    | ++++ | **     | **** | **** |
| Ruta graveolens  | Aerial parts            | --           | +    | ++++ | **     | ***  | ***  |
| Zanthoxylum ailanthoides | Barks     | +            | +    | +++  | **     | **   | **   |
| Zanthoxylum bungeanum | Peels              | --           | +    | +    | **     | **   | **   |
| Sapindaceae       | Aesculus turbinata      | ++           | --   | --   | --     | --   | --   |
| Sapindaceae       | Alangium malabarum      | ++           | --   | --   | --     | --   | --   |
| Dimocarpus longan | Leaves                  | +++          | +    | +++  | --     | --   | --   |
| Litchi chinensis  | Leaves                  | +            | +    | +++  | --     | --   | --   |
| Sapindus makurossi| Peels                   | +++          | +++  | +++  | ****   | **   | **   |
| Sapotaceae        | Chrysophyllum cainito    | Leaves       | +    | +    | NT     | NT   | NT   |
| Schisandraceae    | Kadsura japonica        | Leaves       | --   | --   | +      | --   | --   |
| Schisandraceae    | Schisandra chinensis    | Fruits       | --   | --   | --     | --   | --   |
| Scrophulariaceae  | Scrophularia baergerianna| Roots        | --   | --   | --     | --   | --   |
| Simaroubaceae     | Picrasma quassioides    | Woods        | +    | +    | --     | --   | --   |
| Smilacaceae       | Smilax china            | Rhizomes     | +    | +    | ++     | --   | --   |
| Solanaceae        | Brunfelsia latifolia    | Leaves       | +    | --   | --     | --   | --   |
| Nicandra physalodes| Fruits                | --           | --   | --   | --     | --   | --   |
| Physalis angulata | Aerial parts            | +++          | +++  | +++  | --     | --   | --   |
| Physalis pruinosa | Aerial parts            | ++++         | +++  | +    | ++     | ***  | ***  |
| Solanum mammosum  | Aerial parts            | +            | +    | --   | --     | --   | --   |
| Solanum nigrum    | Aerial parts            | --           | +    | ++   | **     | **   | **   |
| Taxaceae          | Cephalotaxus harringtonia| Leaves      | ++++ | ++++ | +++    | **** | **** |
| Torreya grandis   | Seeds                   | ++           | +    | +++  | --     | --   | --   |
| Theaceae          | Camellia sinensis       | Leaves       | ++   | NT   | NT     | NT   | NT   |
| Thymelaeaceae     | Daphne genkwa          | Flowers      | +    | +    | +      | **   | **   |
Because the effects of compound 23 were extremely weak, this suggested the 3,4-dihydroxyphenethyl group is essential for the observed strong anti-proliferative activity. Furthermore, 3,4-dihydroxyphenethyl alcohol itself showed potent activity [13]. It is also known that treatment of phenylethanoids resulted in apoptotic cell death [16].

Polyphenols (Fig. 4)

Epidemiological studies have suggested that the consumption of green tea [Camellia sinensis (Theaceae)] provides protection against stomach cancer. In a rural area of northern Kyushu, Japan, a decreased risk of stomach cancer was also noted among people reporting a high consumption of green tea [17]. Fractionation of green tea extract, guided by the anti-proliferative activity against MK-1 cells, resulted in the isolation of six flavan-3-ols (24–29) together with the inactive glycosides of kaempferol and quercetin [18]. A study of their structure–activity relationships suggested that the presence of the three adjacent hydroxyl groups (pyrogallol or galloyl group) in the molecule is a key factor for enhancing the compound’s activity. Six active polyphenols (30–35) were isolated from the seeds of Rhynchosia volubilis (Fabaceae) after activity-guided fractionation against MK-1, HeLa, and B16F10 cells [19]. These compounds all showed much stronger inhibition against B16F10 cell growth than against HeLa and MK-1 cell growth. Gallic acid (31) with a free carboxyl group showed higher activity than its methyl ester (32). A hydrolysable tannin (36) and two condensed tannins (37, 38) isolated from Phyllanthus emblica (Phyllanthaceae) also showed potent activity [20] against three cell lines. It was proposed that the anti-cancer properties of polyphenols may be related to their ability to participate in a copper-dependent prooxidant mechanism [21].

Flavones (Fig. 5)

After activity-guided fractionation against MK-1, HeLa, and B16F10 cells, 11 active flavones (39–49) were isolated.

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Table 1 continued

| Family         | Scientific name             | Parts                  | MK-1 | HeLa | B16F10 | MT-1 | MT-2 |
|----------------|-----------------------------|------------------------|------|------|--------|------|------|
| Urticaceae     | Daphne odora                | Roots                  | –    | –    | ++++   | –    | –    |
|                | Edgeworthia chrysantha      | Roots                  | –    | –    | +      | –    | –    |
|                | Cecropia obtusifolia        | Fresh leaves           | +    | –    | +      | –    | –    |
|                |                            | Leaves                 | ++   | +    | +      | –    | –    |
|                | Urtica dioica               | Leaves, Twigs          | –    | –    | –      | –    | –    |
|                | Urtica thunbergiana         | Aerial parts           | –    | –    | +      | –    | –    |
| Verbenaceae    | Aloysia triphylla           | Leaves                 | +++  | +    | +      | –    | –    |
|                | Lantana camara var. aculeata| Leaves                 | +++  | +++  | +      | –    | –    |
|                |                            | Stems                  | +++  | +++  | +      | –    | –    |
|                | Lantana montevidensis       | Leaves                 | ++++ | ++++ | ++++++ | –    | –    |
|                | Lippia canescens            | Aerial parts           | –    | –    | +      | –    | –    |
|                | Lippia dulcis               | Aerial parts           | +    | –    | +++    | –    | –    |
|                | Lippia triphylla            | Leaves                 | +    | –    | +++    | –    | –    |
|                |                            | Stems                  | –    | –    | +      | –    | –    |
|                | Verbena brasiliensis        | Aerial parts           | +    | –    | +      | –    | –    |
|                |                            | Roots                  | +    | –    | ++     | –    | –    |
|                | Verbena officinalis         | Aerial parts           | +    | –    | +      | –    | –    |
|                |                            | Roots                  | –    | –    | –      | –    | –    |
| Vitaceae       | Cayratia japonica           | Aerial parts           | –    | –    | +      | –    | –    |
| Xanthorrhoeaceae| Aloe ferox                  | Leaves                 | +    | –    | –      | –    | –    |
| Zingiberaceae  | Alpinia japonica           | Fruits                 | –    | –    | –      | –    | –    |
|                |                            | Seeds                  | –    | +    | +      | –    | **   |
|                | Curcuma zedoaria            | Rhizomes               | +    | –    | +      | **   | **   |
|                | Hedychium coronarium        | Rhizomes               | +    | +    | +      | **   | **   |
|                | Zingiber officinale         | Rhizomes               | +    | +    | ++     | **   | **** |

The extracts are classified in the Angiosperm Phylogeny Group III system. EC_{50} values against MK-1, HeLa, and B16F10 cells (<3.13 µg/mL, +++++; 3.13–6.25 µg/mL, +++++; 6.25–12.5 µg/mL, ++++; 12.5–25 µg/mL, +++; 25–50 µg/mL, ++; 50–100 µg/mL, +; >100 µg/mL, -). EC_{50} values against MT-1 and MT-2 cells (<0.1 µg/mL, ********; 0.1–1 µg/mL, ******; 1–10 µg/mL, ****; 10–100 µg/mL, **; >100 µg/mL, -)

NT not tested
from the leaves of *Lantana montevidensis* (Verbenaceae) [22]. Concurrently, several related flavones (50–57) isolated from other plant materials and two synthetic ones (58, 59) were also evaluated. 5,7-Dihydroxy flavones (39, 50, 51), 5,7-dihydroxy-6-methoxy flavones (40, 41, 54, 55), and 6-methoxy flavone (59) were much stronger inhibitors of HeLa cell growth than B16F10 and MK-1 cell growth. In particular, compound 59 was a potent inhibitor of HeLa cell growth. Therefore, the 6-methoxy group is likely important for enhancing the anti-proliferative activity of flavones against HeLa cells. A synthetic flavone derivative, flavopiridol (Alvocidib), is being evaluated in clinical trials of ovarian and primary peritoneal cancers [23].

### Sesquiterpenes (Fig. 6)

After activity-guided fractionation against MK-1, HeLa, and B16F10 cells, five active sesquiterpenes (60–64) were isolated from the roots of *Inula helenium* (Asteraceae) together with an inactive sesquiterpene (65) and a weak one (66) [24]. A structure–activity study suggested that the presence of an α-methylene-γ-lactone group is a key component required for the anti-proliferative activity. The thiol reactivity of the α-methylene-γ-lactone group may be responsible for the observed anti-proliferative activity [25]. Two norsesquiterpene glycosides from the roots of *Phyllanthus emblica* (Phyllanthaceae) exhibited potent activity (data not shown) although their aglycone and monogluco-side showed no inhibitory activity [20].

### Triterpene glycosides (Fig. 7) and triterpenes (Fig. 8)

From the bioactive fraction of the fruits of *Bupleurum rotundifolium* (Apiaceae), ten ursane-type triterpene glycosides were isolated and their anti-proliferative activities against MK-1, HeLa, and B16F10 cells were estimated [26]. All active glycosides (67–71) have a 13β, 28-epoxy
| Compound name                                           | MK-1 | HeLa | B16F10 | MT-1 | MT-2 | Normal |
|---------------------------------------------------------|------|------|--------|------|------|--------|
| Falcarindiol (1)                                        | 15   | 149  | 89     | NT   | NT   | NT     |
| Panaxynol (2)                                           | 1.2  | 224  | 80     | NT   | NT   | NT     |
| 8-O-Acetylfalcarindiol (3)                              | 274  | 175  | 203    | NT   | NT   | NT     |
| (9Z,1,9-Heptadecadiene-4,6-diyn-3,8,1 1-triol (4)       | 8    | 106  | 62     | NT   | NT   | NT     |
| Japoangelol A (5)                                       | 15   | 24   | 32     | NT   | NT   | NT     |
| Japoangelol B (6)                                       | 8.7  | 26   | 42     | NT   | NT   | NT     |
| Japoangelol C (7)                                       | 20   | 32   | 28     | NT   | NT   | NT     |
| Japoangelol D (8)                                       | 30   | 41   | 53     | NT   | NT   | NT     |
| Deoxypodophyllotoxin (9)                                | 0.055| 0.082| 0.21   | 0.006| 0.003| NT     |
| (-)-Deoxypodorhizone (10)                              | 1.85 | 3.2  | 4      | NT   | NT   | NT     |
| Nemerosin (11)                                          | 1.8  | 1    | 1.8    | NT   | NT   | NT     |
| Anthriscinol methyl ether (12)                          | 13   | 11   | 11     | NT   | NT   | NT     |
| Elemicin (13)                                           | 22   | 9.6  | 13     | NT   | NT   | NT     |
| Anthriscusin (14)                                       | 6.2  | 5.2  | 7.5    | NT   | NT   | NT     |
| Morelensin (15)                                         | 0.24 | 0.14 | 0.23   | NT   | NT   | NT     |
| (-)-Hinokinin (16)                                      | 4.8  | 7.3  | 7.6    | NT   | NT   | NT     |
| Acteoside (17)                                          | 35   | 50   | 11     | NT   | NT   | NT     |
| Isoacteoside (18)                                       | 40   | 32   | 10     | NT   | NT   | NT     |
| Arenarioside (19)                                       | 34   | 34   | 16     | NT   | NT   | NT     |
| Leucosceptoside A (20)                                  | 42   | 33   | 28     | NT   | NT   | NT     |
| Ligupurpuroside A (21)                                  | 26   | 69   | 6.5    | NT   | NT   | NT     |
| Ligupurpuroside C (22)                                  | 49   | 49   | 11     | NT   | NT   | NT     |
| Ligupurpuroside B (23)                                  | >135 | >135 | >135   | 120  | NT   | NT     |
| Epicatechin (24)                                        | 45   | NT   | NT     | NT   | NT   | NT     |
| Epigallocatechin (25)                                   | 14   | NT   | NT     | NT   | NT   | NT     |
| Epigallocatechin gallate (26)                           | 9    | NT   | NT     | NT   | NT   | NT     |
| Gallocatechin (27)                                      | 14   | NT   | NT     | NT   | NT   | NT     |
| Epicatechin gallate (28)                                | 14   | NT   | NT     | NT   | NT   | NT     |
| Gallocatechin gallate (29)                              | 10   | NT   | NT     | NT   | NT   | NT     |
| 7-O-Galloyl catechin (30)                               | 41   | 38   | 9      | NT   | NT   | NT     |
| Gallic acid (31)                                        | 19   | 22   | 7.1    | NT   | NT   | NT     |
| Gallic acid methylester (32)                            | 65   | 43   | 18     | NT   | NT   | NT     |
| Trigalloylgallic acid (33)                              | 10   | 9.3  | 2.9    | NT   | NT   | NT     |
| 1-O-Galloylgallose (34)                                 | 60   | 45   | 15     | NT   | NT   | NT     |
| 1,6-Di-O-galloylgallose (35)                            | 39   | 29   | 8.1    | NT   | NT   | NT     |
| Corilagin (36)                                          | 13   | 30   | 4.7    | NT   | NT   | NT     |
| Prodelphidin B1 (37)                                    | 13   | 15   | 3.3    | NT   | NT   | NT     |
| Prodelphidin B2 (38)                                    | 15   | 15   | 3.3    | NT   | NT   | NT     |
| Apigenin (39)                                           | 22   | 15   | 26     | NT   | NT   | NT     |
| Hispidulin (40)                                         | 83   | 17   | 67     | NT   | NT   | NT     |
| Eupafolin (41)                                          | 29   | 6    | 16     | NT   | NT   | NT     |
| Compound 42                                             | 55   | 55   | 18     | NT   | NT   | NT     |
| Compound 43                                             | 73   | 73   | 29     | NT   | NT   | NT     |
| Compound 44                                             | 33   | 44   | 39     | NT   | NT   | NT     |
| Cirsiliol (45)                                          | 18   | 21   | 9      | NT   | NT   | NT     |
| Eupatorin (46)                                          | 58   | 15   | 44     | NT   | NT   | NT     |
| Cirsilineol (47)                                        | 17   | 203  | 73     | NT   | NT   | NT     |
| Compound 48                                             | 22   | 14   | 14     | NT   | NT   | NT     |
| Compound name | MK-1 | HeLa | B16F10 | MT-1 | MT-2 | Normal |
|---------------|------|------|--------|------|------|--------|
| Chrysin (50)  | 63   | 8    | 51     | NT   | NT   | NT     |
| Luteolin (51) | 31   | 10   | 21     | NT   | NT   | NT     |
| Baicalein (52)| 26   | 30   | 11     | NT   | NT   | NT     |
| 6-Hydroxyluceolin (53)| 26 | 30 | 13 | NT | NT | NT |
| Pectolinarigenin (54) | 115 | 10 | 64 | NT | NT | NT |
| Desmethoxycentauredin (55) | 24 | 9 | 64 | NT | NT | NT |
| Jaceosidin (56) | 27 | 33 | 27 | NT | NT | NT |
| Eupatilin (57) | 55 | 35 | 58 | NT | NT | NT |
| 7-Methoxyflavone (58) | 119 | 87 | 119 | NT | NT | NT |
| 6-Methoxyflavone (59) | 398 | 8 | 398 | NT | NT | NT |
| 1,3,11(13)-Elematrien-8β, 12-olide (60) | 6.9 | 13 | 4.3 | NT | NT | NT |
| 5α-Epoxyalantolactone (61) | 6.9 | 6.5 | 3.6 | NT | NT | NT |
| 4β,5α-epoxy-1(10),11(13)-germacradiene-8,12-olide (62) | 12 | 33 | 14 | NT | NT | NT |
| Alantolactone (63) | 6.9 | 6.9 | 4.7 | NT | NT | NT |
| Isoalantolactone (64) | 44 | 41 | 29 | NT | NT | NT |
| 11α,13-Dihydroalantolactone (65) | >427 | >427 | >427 | NT | NT | NT |
| 11α,13-Dihydroisoalantolactone (66) | >427 | >427 | >427 | 44 | NT | NT |
| Rotundifolioside I (67) | 20 | 37 | 18 | NT | NT | NT |
| Rotundifolioside J (68) | 16 | 21 | 11 | NT | NT | NT |
| Rotundifolioside A (69) | 48 | 71 | 31 | NT | NT | NT |
| Rotundifolioside H (70) | 18 | 31 | 18 | NT | NT | NT |
| Rotundifolioside G (71) | 84 | >108 | 46 | NT | NT | NT |
| Rotundifolioside E (72) | >110 | >110 | >110 | NT | NT | NT |
| Rotundifolioside F (73) | >108 | >108 | >108 | NT | NT | NT |
| Rotundioside F (74) | 17 | 19 | 6.6 | NT | NT | NT |
| Rotundioside G (75) | 7.8 | 15 | 17 | NT | NT | NT |
| Rotundioside T (76) | 13 | 12 | 7.7 | NT | NT | NT |
| Rotundioside Q (77) | 34 | 37 | 12 | NT | NT | NT |
| Rotundioside S (78) | 19 | 34 | 8.9 | NT | NT | NT |
| Ursolic acid lactone (79) | 90 | 88 | 194 | NT | NT | NT |
| Ursolic acid (80) | 19 | 65 | 14 | NT | NT | NT |
| Pomolic acid (81) | 55 | 59 | 29 | NT | NT | NT |
| Corosolic acid (82) | 59 | 69 | 44 | NT | NT | NT |
| 2α,3α-Dihydroxy-urs-12-en-28-oic acid (83) | 55 | 38 | 36 | NT | NT | NT |
| 3-Epipasminic acid (84) | 21 | 21 | 19 | NT | NT | NT |
| Psoralen (85) | 403 | 40 | 376 | 345 | 177 | NT |
| Bergapten (86) | 167 | 37 | 167 | 189 | 214 | NT |
| Xanthotoxol (87) | >431 | 16 | 289 | NT | NT | NT |
| 8-Hydroxybergapten (88) | 139 | 8.9 | 104 | NT | NT | NT |
| Xanthotoxin (89) | 139 | 74 | 181 | 73 | 48 | NT |
| Isopimpinellin (90) | 151 | 53 | 159 | 85 | 231 | NT |
| 1,3-Dihydroxy-4-[(2'-hydroxy-3'-hydroxymethyl-3',4'-epoxybutyl)-N-methylation (91)] | 0.056 | 0.056 | 1.76 | NT | NT | NT |
| 1,3-Dihydroxy-4-{[(Z)-3'-hydroxy-3'-methylbuten-1'-yl]-N-methylation (92)] | 308 | 68 | 13 | NT | NT | NT |
| 4β-Hydroxywithanolide E (93) | NT | NT | NT | 0.2 | 0.2 | 1.6 |
| withanolide S (94) | NT | NT | NT | 196 | 81 | NT |
### Table 3 continued

| Compound name | MK-1 | HeLa | B16F10 | MT-1 | MT-2 | Normal |
|---------------|------|------|--------|------|------|--------|
| 5α-O-Methylwithanolide S (95) | NT | NT | NT | 21 | 3.6 | NT |
| 5α-O-Butylwithanolide S (96) | NT | NT | NT | 2.4 | 0.8 | NT |
| 2-Hydro-3β-methoxy-4β-hydroxywithanolide E (97) | NT | NT | NT | 1.9 | 1.7 | NT |
| Sitoinoside IX (98) | NT | NT | NT | 0.83 | 6.1 | NT |
| Withaferine A (99) | NT | NT | NT | 0.16 | 1.3 | NT |
| 2,3-Dihydrowithaferine A (100) | NT | NT | NT | 0.022 | 0.51 | NT |
| 24,25-Dihydrowithanolide D (101) | NT | NT | NT | 0.008 | 0.008 | 860 |
| Physapruin A (102) | NT | NT | NT | 0.05 | 0.28 | NT |
| Withanolide F (103) | NT | NT | NT | 1.4 | 1.6 | NT |
| Nivaphysalin A (104) | NT | NT | NT | >100 | >100 | NT |
| Nivaphysalin B (105) | NT | NT | NT | >100 | >100 | NT |
| Nivaphysalin C (106) | NT | NT | NT | 59 | 58 | NT |
| Liriodenine (107) | NT | NT | NT | 3.1 | 3.6 | NT |
| Lysicamne (108) | NT | NT | NT | 32 | 16 | NT |
| Lanuginosine (109) | NT | NT | NT | 1.3 | 4.5 | NT |
| 14β-Hydroxytylophorine N-oxide (110) | NT | NT | NT | 0.07 | 0.027 | NT |
| Tylophorine N-oxide (111) | NT | NT | NT | 0.029 | 0.0048 | NT |
| 3-Demethyl-14β-hydroxyisotylocrebrine N-oxide (112) | NT | NT | NT | 0.0083 | 0.0071 | 0.04 |
| Tylophorine N-oxide (113) | NT | NT | NT | 1.6 | 1.5 | NT |
| Isotylocrebrine N-oxide (114) | NT | NT | NT | 0.38 | 0.25 | NT |
| 3-Demethyl-14β-hydroxyisotylocrebrine (115) | NT | NT | NT | 0.0028 | 0.0026 | NT |
| Tylophorine (116) | NT | NT | NT | 0.076 | 0.051 | NT |
| Isotylocrebrine (117) | NT | NT | NT | 0.048 | 0.025 | NT |
| 7-Demethyltylophorine (118) | NT | NT | NT | 0.019 | 0.029 | NT |
| 5-FU | 21 | 13 | 1.1 | NT | NT | NT |
| DOX | NT | NT | NT | 0.015 | 0.013 | NT |

*Dox* doxorubicin, *5-FU* 5-fluorouracil (positive controls), *NT* not tested

**Fig. 2** Structures of lignans identified from a screen of plant extracts
ring system in the molecule except for 72 and 73, which have a 21β-hydroxy group. The glycosides of the other aglycones are almost inactive. Among the active glycosides, 69 and 71, which have a glucosyl group directly linked to the aglycone instead of a fucosyl group, were less potent. It is possible that the fucosyl group plays some role in the anti-proliferative activity. From the same fraction, 19 oleanane-type triterpene glycosides were also isolated and their anti-proliferative activities were evaluated [27]. Similar to the ursane-type triterpene glycosides, all active glycosides (74–78) have a 13β, 28-epoxy ring system in the molecule. In contrast to the ursane-type triterpene glycosides, compounds 77 and 78, which have a 21α-hydroxy group, had potent anti-proliferative activities. The configuration at the C-21 hydroxy group might influence the anti-proliferative activity.

After activity-guided fractionation against MK-1, HeLa, and B16F10 cells, ten triterpenes were isolated from the aerial parts of *Centella asiatica* (Apiaceae) [28]. Some (79–84) of these triterpenes showed potent anti-proliferative activities. Similar to the results of the polyphenols, ursolic acid (80) with a free carboxyl group showed higher activity than its lactone (79). Ursolic acid (80) was previously reported to induce apoptotic cell death [29].

**Coumarins and acridone alkaloids (Fig. 9)**

After activity-guided fractionation against MK-1, HeLa, and B16F10 cells, 16 compounds were isolated from the aerial parts and roots of *Boenninghausenia japonica* (Rutaceae) [30]. Among them, an acridone alkaloid (91) showed very strong anti-proliferative activity against these three cell lines. The EC50 value of 91 was in the nanomolar range except for against B16F10 cells. Therefore, a 3′, 4′-epoxy group might be important for enhancing the anti-proliferative activity of acridone alkaloids. Furthermore, some furanocoumarins (85–90) showed potent anti-proliferative activities against HeLa cells. The furanocoumarins (87, 88) having an 8-hydroxy group showed more potent activity than those without the substituent (85, 86) and those with an 8-methoxy group (89, 90) against HeLa cells. Therefore, an 8-hydroxy group may be important for enhancing the anti-proliferative activity of these compounds against HeLa cells. Some furanocoumarins (85, 86,
89, 90) also showed moderate anti-proliferative activity against MT-1 and MT-2 cells (Table 3). A recent review reports that natural and synthetic coumarins have anti-cancer activity toward various cell lines [31].

Withanolides (Fig. 10)

After activity-guided fractionation against MT-1 and MT-2 cells, five active withanolides (93–97) were isolated from the aerial parts of Physalis pruinosa (Solanaceae) [32]. Structure–activity relationships suggested that the presence of a 5β, 6β epoxy group in the B-ring and a 4β-hydroxy group in the A-ring were important for the observed activities. The aliphatic ether side chain at C-5 also seems to increase the activity because as the side chain is lengthened, the activity increases. Because the EC$_{50}$ value for 4β-hydroxywithanolide E (93) was in the nanomolar range against both MT-1 and MT-2 cells, 31 other withanolides were also evaluated [33]. Except for compound 98, none of the glycosides showed any activity against the ATL cell lines. Because compound 98 has a 5β, 6β-epoxy group as well as a 4β-hydroxy group, we predicted it might show potent activity. However, the activity of the corresponding deglycosylated compound (99) was approximately four times greater than that of its glucoside (98). These results indicated that the presence of a sugar moiety should reduce the anti-proliferative effects. The importance of the 5β, 6β-epoxy group, and 4β-hydroxy group was further supported by the analysis of compound 100 because it showed the second strongest anti-proliferative activity. The activities of 99, having a double bond between C-2 and C-3, were weaker than those for 100, suggesting the double bond between C-2 and C-3 might reduce the activity. Because compound 102 showed greater activity when compared with compound 103, the importance of a 4β-hydroxy group was further supported. Compared with the compounds having the 5β, 6β-epoxy group, the activities of the compounds (104–106) having other types of epoxy groups (6α, 7α-epoxy group in the B-ring and/or 24α, 25α-epoxy group in the E-ring) were significantly lower. The position of the epoxy group and/or the configuration of the epoxy group seem to be important for the activity. Compound 106 containing a 15β-hydroxy group showed moderate activity while compound 105 containing a 15α-
hydroxy group did not show any activity. This indicated that the configuration of the hydroxy group at C-15 may influence a compound’s activity. Finally, the EC$_{50}$ value of 24, 25-dihydrowithanolide D (101), the most potent withanolide-type inhibitor, was 8 nM against both cells. In contrast, the cytotoxic effect (860 nM) of 101 toward normal cells was observed at a concentration about 100 times higher than those observed for the ATL cell lines. Furthermore, compound 101 was confirmed to induce dose-dependent apoptosis against MT-1, MT-2, and fresh ATL cells [33]. Therefore, 24, 25-dihydrowithanolide D (101) may be a promising chemotherapeutic candidate.

Recently inhibition of the growth of human lung cancer cells through DNA damage, apoptosis and G2/M arrest by 4β-hydroxywithanolide E (93) have been reported [34]. Further, induction of apoptosis in leukemia cells by targeting the activation of a neutral sphingomyelinase-ceramide cascade mediated by synergistic activation of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase by withanolide D have been also reported [35].
Fig. 7 Structures of triterpene glycosides identified from a screen of plant extracts

rotundifolioside I (67): \( R = \text{Fuc}^2-\text{Glc}^2-\text{Xyl} \)
rotundifolioside J (68): \( R = \text{Fuc}^2-\text{Glc}^2-\text{Rha} \)
rotundifolioside A (69): \( R = \text{Glc}^2-\text{Glc}^2-\text{Xyl} \)
rotundifolioside H (70): \( R = \text{Fuc}^2-\text{Glc}^2-\text{Xyl} \)
rotundifolioside G (71): \( R = \text{Glc}^2-\text{Glc}^2-\text{Xyl} \)
rotundifolioside E (72): \( R = \text{Fuc}^2-\text{Glc}^2-\text{Xyl} \)
rotundifolioside F (73): \( R = \text{Fuc}^2-\text{Glc}^2-\text{Rha} \)
rotundifolioside G (75): \( R = \text{Glc}^2-\text{Glc}^2-\text{Xyl} \)

rotundidioside T (76): \( R = \text{Fuc}^2-\text{Glc}^2-\text{Xyl} \)
rotundidioside Q (77): \( R = \text{Fuc}^2-\text{Glc}^2-\text{Xyl} \)
rotundidioside S (78): \( R = \text{Fuc}^2-\text{Glc}^2-\text{Rha} \)

Fig. 8 Structures of triterpenes identified from a screen of plant extracts

ursolic acid lactone (79)
ursolic acid (80)
pomolic acid (81)
corosolic acid (82)
2α,3α-dihydroxy-urs-12-en-28-ol acid (83)
3-epimaslinic acid (84)
Fig. 9  Structures of coumarins and acridone alkaloids identified from a screen of plant extracts

- psoralen (85)
- xanthotoxol (87)
- xanthotoxin (89)
- bergapten (86)
- 8-hydroxybergapten (88)
- isopimpinellin (90)
- 1,3-dihydroxy-4-(2'-hydroxy-3'-hydroxymethyl-3',4'-epoxybutyl)-N-methylacridone (91)
- 1,3-dihydroxy-4-[(Z)-3'-hydroxy-3'-methylbuten-1'-yl]-N-methylacridone (92)

Fig. 10  Structures of withanolides identified from a screen of plant extracts

- 4β-hydroxywithanolide E (93)
- withanolide S (94): R = H
- 5α-O-methylwithanolide S (95): R = CH₃
- 5α-O-butylowithanolide S (96): R = n-Butyl
- 2-hydro-3β-methoxy-4β-hydroxywithanolide E (97)
- sitosidoside IX (98): R = Glc
- withaferine A (99): R = H
- 2,3-dihydrowithaferine A (100)
- 24,25-dihydrowithanolide D (101)
- physapruin A (102): R = OH
- withanolide F (103): R = H
- nivaphysalin A (104)
- nivaphysalin B (105): 15α
- nivaphysalin C (106): 15β
Wang et al. suggested that Hsp90 inhibition by the withanolides is correlated with their ability to induce cancer cell death [36].

**Aporphine and phenanthroindolizidine alkaloids**  
(Fig. 11)

After activity-guided fractionation against MT-1 and MT-2 cells, three active aporphine alkaloids (107–109) were isolated from the leaves of *Annona reticulata* and *A. squamosa* (Annonaceae) [37]. Liriodenine (107) showed accumulation of Sub-G1 stage cells in the MT-1 and MT-2 cell population, suggesting induction of apoptosis. A structure–activity relationship analysis suggested that the presence of a 1, 2-methylenedioxy group seemed to enhance activity. A similar conclusion on the structure–activity relationship was also obtained by Liu et al. [38].
Six phenanthroindolizidine alkaloids (110–115) were isolated from the aerial parts of *Tylophora tanakae* (Asclepiadaceae) by activity-guided fractionation [39]. In addition to 110–115, three phenanthroindolizidine alkaloids (116–118) obtained from other plants were examined for their anti-proliferative activity against MT-1 and MT-2 cells. The EC50 values of all alkaloids except for compound 113 were in the low nanomolar range. The results suggested that the presence of a 2-methoxy functionality, the methyl group of a 7-methoxy functionality, and an N-oxide moiety appear to reduce the potency of the anti-proliferative activity [39]. Phenanthroindolizidine alkaloids are cytotoxic to multidrug-resistant cells [40], inhibiting the enzyme dihydrofolate reductase [41]. The in vivo efficacy of a new phenanthroindolizidine alkaloid derivative (YPC-10157) was recently evaluated [42].

**Conclusions**

Cytotoxicity against selected cancer cell lines was characterized and could be explained by identifying the active principles responsible for the observed effects. The polyacetylenes was more potent against MK-1 cells than against HeLa and B16F10 cells. The EC50 value of the most potent polyacetylene (2) against MK-1 cells was 1.2 μM (Fig. 12). The compounds (17–22) having a 3, 4-dihydroxyphenethyl group also showed remarkable anti-proliferative effects against B16F10 cells (Fig. 12). Interestingly, some 6-methoxyflavone derivatives (40, 41, 54, 55, 59) and 8-hydroxy furanocoumarins (87, 88) showed strong inhibition against HeLa cell growth (Fig. 12).

The compounds whose EC50 values were less than one nanomolar (<1 nM) were not selective for specific cell types. This group included two lignans (9, 15), one acridone alkaloid (91), six withanolides (93, 98–102), and eight phenanthroindolizidine alkaloids (110–112, 114–118). Because the cytotoxic effect of 24, 25-dihydrowithanolid D (101) toward normal cells was observed at a concentration about 100 times higher than against the ATL cell lines, withanolide was concluded to be the most promising chemotherapeutic candidate from our experiments.

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