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Preparation of Philippine Plant Extract Libraries for High-Throughput Screening

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ABSTRACT

Various terrestrial plants were collected from different places in the Philippines. The dried samples were then soaked in methanol and partitioned using modified Kupchan method. Portions of the ethyl acetate and aqueous fractions were treated for the removal of polyphenols. Dried methanolic, hexane, ethyl acetate, aqueous fractions and portions treated for polyphenols were weighed and dissolved in dimethyl sulfoxide to give a concentration of 5mg/ml. Philippine Plant Library I, with 200 wells and Philippine Plant Library II, which has 628 wells, are currently deposited at the Institute of Chemistry and Cell Biology Screening Facility at Harvard Medical School are continuously being subjected to different assays.

Keywords: natural products, plant extracts, high-throughput screen

INTRODUCTION

Terrestrial plants have been a major source of therapeutic agents used for the cure of human ailments. A considerable number of drugs have been obtained from plants, which are used for a wide range of therapeutic activities. These include steroids, analgesics, antihypertensives, cholinergics, antimalarials, antigout and anticancer agents (1–5). Scientists continue to sift through this reservoir of molecules looking for the inimitable compound, which may serve as a research tool, a new potential drug or drug-prototype (6–8).

One of the new tools used to identify potential therapeutic agents is the cellular and subcellular "mechanism-based" high throughput screening assays (9). Compounds are tested for a wide range of biological activities ranging from cytotoxicity to antibacterial, antiviral and anti-inflammatory activity. These tests are carried out with little human intervention and 'robots' report the positive hits automatically for further examination. The compounds derived from three main sources: natural materials – plants, microorganism and animals, regular chemical synthesis and combinatorial chemistry (10–12).

Combinatorial libraries based on natural products template are excellent starting points for diversification since these compounds were honed by their evolutionary history for biological activity (13). The objective of this study was to prepare a library of plant extracts which can be subjected to high throughput screening and could be a source of templates for compound library construction.

MATERIALS AND METHODS

Plants

Plants were collected from Laguna, Quezon City and Tarlac, Philippines. These plants were submitted for

identification to the Dr. Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman.

Chemicals

The methanol used for soaking was technical grade. The hexane and ethyl acetate used for extraction were also technical grade and singly distilled. Polyphenols were removed using polyvinylpyrollidone (Sigma Aldrich).

Extraction

The dried samples were cut into pieces for overnight soaking in MeOH at room temperature. The resulting extract was concentrated in vacuo. Water was then added to the methanol concentrate to make 90% solution, which was then defatted with hexane. Additional water was added to the alcoholic fraction (c.a. 60%), which was then consecutively partitioned with EtOAc.

Tannin removal

Dried aqueous extracts (50mg) were dissolved in water (15ml) and ethyl acetate extracts in methanol-water. Polyvinylpyrollidone (1.25g) was added and the resulting mixture vortexed and centrifuge. The supernatant was withdrawn and dried.

EtOAC plant extracts are concentrated to a solid residue and aqueous extracts freeze dried. Both samples (10mg) were treated with hot distilled deionized water (6ml) and filtered if necessary. The solution was divided into three parts. To the first 1% NaCl solution was added, to the second 1% NaCl and gelatin were added. Formation of a precipitate in the second indicates the presence of tannins, which is confirmed by the appearance of blue, blue-black or blue-green color on addition of $FeCl_3$ solution to a third portion. The steps are repeated until the test for tannins is negative (14).

DISCUSSION

The dried methanolic, hexane, ethyl acetate, aqueous portions and those portions treated for polyphenols were weighed and dissolved in dimethylsulfoxide to give a concentration of 5mg/ml. Philippine Plant Library I, with 200 wells and Philippine Plant Library II, which has 628 wells as shown in Figure 1 were deposited at the Institute of Chemistry and Cell Biology Screening Facility at Harvard Medical School. These libraries are now part of the ICCB's compound collection (www.iccb.med.harvard.edu). The ICCB develops and uses automation to test large collections of molecules in a wide variety of biological assays. This set-up assists scientists to discover and elucidate molecular pathways fundamental to cell and disease biology. Some of the screens identify compounds that 1) affect cytokinesis in Drosophila culture cells, 2) inhibit invasion or growth of Plasmodium falcifarum in whole cell assay, 3) affect cell cycle progression of synchronized HeLa cells, 4) inhibit XKCMI microtubule depolymerizing activity, and 5) overide the mitotic checkpoint in HeLa cells treated with taxol.

Most of the hits from the Philippine plant extract libraries were extracts prepared from plants with folkloric basis. Screens showed Artemisia vulgaris and Hopea acuminata as positive in the screen that affect cell cycle progression of synchronized HeLa cells. A. vulgaris is a commonly used medicinal plant in the Philippines for various conditions like expectorant, stomachic, antispasmodic and anthelmintic (15). Blumea balsamifera, Cassia alata, and Chrysanthemum indicum were positive in the assay that inhibits XKCMI microtubule depolymerizing activity. B. balsamifera is another common medicinal plant found throughout the Philippines and used for colds, dissolution of kidney stones, anti-diarrheal (15). C. alata is used traditionally for skin diseases, expectorant and astringent (15). C. indicum is used as a carminative (15). The anti-mitotic component of H. acuminata has been isolated and identified as the stilbene ampelopsin B (16). The bioactive compounds that will be isolated from these hits can be used in the future to build natural-product like libraries which could lead to potentially more active compounds.

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