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7. Title
Natural Drug Discovery from Medicinal Plants

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Agricultural Experiment Sta, Louisiana State Univ

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	10/01/2012	09/30/2016

Goals/Objectives/Expected Outputs

GOAL: Isolate and identify bioactive compounds from medicinal plants. SPECIFIC OBJECTIVES: 1. Develop effective extraction and purification methods for plants that have promising bioactivity in improving human health (e.g., prevention and treatment of cancer; weight loss, diabetes; and antimicrobial). 2. Screen medicinal plant extracts and isolated natural compounds for relevant bioactivity (e.g., anti-cancer). 3. Elucidate the structures of natural compounds that show promising bioactivities. EXPECTED OUTCOMES: Definitive understanding of the bioactivity and its responsible components of plant extracts. These knowledge will support more in depth investigations toward natural drug development.

Methods

Plant extract preparations The Medicinal Plant Laboratory is equipped with state of the art equipment that gives us the flexibility to design, on a case by case basis, the most effective extraction methods. The soxhlet apparatus allows repeated extractions in a low solvent volume and fuller recovery of extractable constituents. The low and high pressure extractors provide rapid extraction of plant materials based on the physicochemical properties of predicted constituents. The re-invented simultaneous blending/extracting procedures have been shown to shorten the extraction time (minutes) yet achieve the same extraction results over the conventional maceration method (days). Due to the vast plant resources, careful selection of those that have promising characteristics for the focused disease conditions will be made benefiting from the knowledge and experience accumulated over the years and shown in high probability of successes in finding the "hit" plants. Isolation of bioactive compounds and structural elucidation Chromatographic columns ranging in size from grams to kilograms of adsorbent materials are used to accommodate various quantities of plant extracts. Among these, two flash chromatographic columns (low-pressure chromatography), one manual and the other with automatic fraction collectors, have been found to be effective and efficient in the isolation of natural compounds of diverse structures. On the analytical side, the laboratory has two HPLC systems with photodiode array (PDA), ELS (evaporative light scattering), and MS (mass spectrometry) detection capabilities. The nearby chemistry core facilities provide services on Nuclear Magnetic Resonance (NMR) and sophisticated MS chromatograms to aid in structural elucidation. Solubility enhancement and bioactivity screening We will first perform solubility enhancement for these poorly soluble samples with natural solubilizers and/or DMSO as the control. Bioactivity screening will begin with cytotoxicity assays that will include an appropriate positive control, for example, curcumin (Zhang et al. 2011). Human cancer cell lines such as prostate carcinoma (PC3), breast carcinoma (MDA-MB-231), and colon adenocarcinoma (HT-29) cell lines will be used. These cells are available from the LSU AgCenter's cell culture core laboratory. In vitro cytotoxicity assays will be conducted using the MTS assay.

HT-29, PC3, and MDA-MB-231 cells are added to 96-well plates at 10000 cells/well, respectively, and allowed to adhere overnight. The cells are then treated with various extracts or isolated natural compounds solutions in triplicate wells and incubated at 37 degree C for 72 hrs. Absorbance is then measured at a wavelength of 490 nm using a Bio-Rad Microplate Reader (Hercules, CA). Percent viability is calculated as cell viability relative to vehicle-treated control (100%). ANOVA with repeat measure analysis if applicable will be used to analyze the data using SAS software. Different treatment means will be separated using Tukey's test. Differences will be regarded significant at P = or < 0.05.

23. Non-Technical Summary

Numerous studies have examined forest resources and other plant ecosystems for bioactive compounds that can be used in the treatment of human illnesses. In fact, over 80% of our current drugs have plant-based origins (Newman et al. 2003). Over the last 12 years the LSU AgCenter's Medicinal Plant Laboratory has conducted over ten projects that have led to the development of a protocol to identify and isolate bioactive compounds from a diversity of forest plants (Liu 2008). The laboratory has been designed to efficiently conduct medicinal plant research, and has leveraged previous McIntire-Stennis support to receive three National Institutes of Health research grant awards (Yin et al. 2008; 2009; Cefalu et al. 2009). This research has had effective results and the potential to discover additional bioactive compounds. In addition, the Medicinal Plant Laboratory also has developed the capability of conducting cell culture and screening for cytotoxicity against human cancer cell lines, as well as animal studies in pharmacokinetics and disease conditions (e.g., obese model). This has become a routine operation and driven our search for additional sources of natural drugs from plants typically found in forested ecosystems. Once identified, promising agents can then be subjected to further investigations of the pharmacological mechanisms underlying their effectiveness, setting the stage for preclinical and translational studies. Sample preparation issues prior to testing often arise while working with plant-related bioactive compounds. This often results from poor solubility of plant extracts prepared with organic solvents (e.g., methanol, ethanol, acetone, or hexane). Solubility issues can be somewhat mitigated by using co-solvents such as ethanol, DMSO, PEG400, or surfactants such as Tween 80 and Labrasol, which are readily available. However, toxicity (activity) stemming from the use of these solubilizing agents may confound the efficacy screening. Alternative to these typical techniques, however, poor solubility can be alleviated by the use of natural compounds. For example, by using a common food sweetener (steviol glycoside), solubility can be remarkably enhanced (Zhang et al., 2011; Jeansonne et al. 2011). This type of research finding has the potential to greatly facilitate accurate screening of medicinal plant extracts and their isolated compounds. We will introduce our newly acquired expertise into our natural drug discovery project to enable more accurate bioactivity screening.

24. Keywords

Plant extracts; Natural compounds; Structural elucidation; Solubility enhancement; Cytotoxicity

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