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Bioassay-Guided Fractionation of Extracts from Biological Sources

Biological sources such as plants, animals, microbes, insects, lichens, etc. produce a range of structurally diverse organic compounds known as natural products, which include acetogenins, terpenoids, flavonoids, quinones, saponins and alkaloids. Some natural products have useful properties and can serve as the basis for the production of pharmaceuticals, agrochemicals, cosmetics, food flavours and colouring agents. Natural products have, for example, been a primary source of commercial medicines and drug leads. The isolation of bioactive compounds from biological sources is a preliminary step in the exploitation of such compounds for human needs.

Bioassay-guided fractionation is an effective protocol for the isolation of active compounds from natural sources, and consists of the following steps: (1) Prepare extracts typically by solvent extraction of natural materials, and assay the extracts for the desired bioactivity employing an appropriate *in vitro* or *in vivo* bioassay; (2) Fractionate the compounds in the bioactive extract based on differences in their physical and chemical properties, usually by solvent partitioning and/or chromatography, and assess the bioactivity of the fractions, (3) Fractionate the active fraction followed by bioassay of the sub-fractions, and repeat this step until pure bioactive natural products are obtained.

Chromatography, the most common method of fractionation employed in bioassay-guided fractionation, is a physical method where the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction. Thin-layer chromatography (TLC), open-column chromatography, flash chromatography, medium-pressure liquid chromatography (MPLC), high-performance liquid chromatography (HPLC) and vacuum-liquid

chromatography (VLC) are some examples of chromatographic methods in which the mobile phase is a liquid and the stationary phase is composed of a solid adsorbent. Counter-current chromatography (CCC) is an all-liquid separation technique and does not involve a solid adsorbent; thus, the irreversible loss or denaturation of active compounds is minimized in CCC. Ion exchange chromatography uses a charged stationary phase to separate charged compounds and involves an ion exchange mechanism. Affinity chromatography is based on selective non-covalent interaction between an analyte and specific molecules which are covalently coupled to an inert matrix such as a polysaccharide bead. Gel filtration chromatography, also known as gel permeation chromatography or size exclusion chromatography, separates molecules according to their size.

In this presentation, extraction procedures and chromatographic methods used in the bioassay-guided fractionation of extracts from biological sources will be discussed. The protocol for isolating bioactive natural products will be further illustrated by taking examples from the literature on the isolation of various bioactive compounds from microorganisms, marine organisms and terrestrial plants.