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Review

Discovery of active components in herbs using chromatographic separation coupled with online bioassay

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ABSTRACT

Discovery of bioactive compounds from complex mixtures is a challenge. In past decades, several strategies were developed and implemented for rapid and effective screening and characterization of bioactive components in complex matrices. This review mainly focused on the online strategies, which integrated the separation science, mass spectrometry, and bioactivity screening in a single platform, allowing simultaneous screening and characterization of active compounds from complex matrices, especially from the herbs. The online screening methodologies, including pre-column affinity-based screening and post-column bioassay, were discussed and their applied examples were also presented to illustrate the strengths and limitations of these approaches.

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Abbreviations: ABTS 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; AChBP, acetylcholine binding protein; AChE, acetylcholinesterase; ACE, angiotensin-converting enzyme; ACN, acetonitrile; APCI, atmospheric pressure chemical ionization; CE, capillary electrophoresis; CHPLC, capillary high performance liquid chromatography; COX, cyclooxygenase; Cyto P450, cytochrome P450; DHFR, dihydrofolate reductase; DAD, diode array detector; EID, enzyme inhibition detection; ESI, electrospray ionization; ER, estrogen receptor; FAC, frontal affinity chromatography; FLD, fluorescence detector; FTICR-MS, fourier transform ion cyclotron resonance mass spectrometry; GC/MS, gas chromatography/mass spectrometry; GST, glutathione-*S*-transferase; HSA, human serum albumin; hER, human estrogen receptor; HPLC, high performance liquid chromatography; HSCCC, high-speed counter-current chromatography; HTLC, high temperature liquid chromatography; HRS, high-resolution screening; HTS, high-throughput screening; IR, infrared; IT-TOF-MS, ion trap time-of-flight mass spectrometry; LED, lightemitting diode; LIF, laser-induced fluorescence; MeOH, methanol; MS, mass spectrometry; NMR, nuclear magnetic resonance; PBS, phosphate buffer salt solution; PDE, phosphodiesterase; PHPLC, preparative high performance liquid chromatography; Q-TOF-MS, quadrupole time-of-flight mass spectrometry; RAM, restricted access material; RAD, receptor affinity detection; RT, retention time; SEC, size exclusion chromatography; SFEID, segmented flow enzyme inhibition detection; SPE, solid phase extraction; SPR, surface plasmon resonance; TCMs, traditional Chinese medicines; TFC, turbulent flow chromatography; TLC, thin layer chromatography; TOF-MS, time-of-flight mass spectrometry; 2DLC, two-dimensional liquid chromatography; UPLC, ultra performance liquid chromatography; UV, ultraviolet; Vis, visible; XOD, xanthine oxidase.

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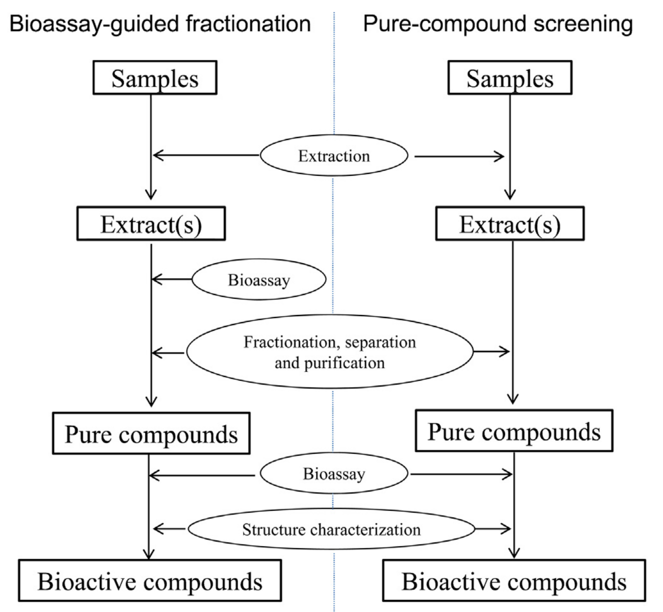

Fig. 1. Scheme of the steps of bioassay-guided fractionation and pure-compound screening.

1. Introduction

Natural products have been recognized as one of the most excellent pool of bioactive compounds for discovering new molecular entities drugs because of their untapped chemical diversity and biological relevance [1]. It was reported that more than half (64%) of 1073 small-molecule new chemical entities drugs introduced between 1981 and 2010 were natural products, natural products derived compounds, synthetic natural product analogues or natural mimic compounds [2].

For the discovery of active compounds from complex mixture, the coexistence of non-active constituents with variant contents is a major obstacle [3]. There are two commonly used approaches leading to the discovery of bioactive compounds from natural products: bioassay-guided fractionation and pure-compound screening. Their essential steps in the search for bioactive compounds are shown in Fig. 1. It is obvious that these processes are very laborious and time-consuming. In addition, the active components could be lost during the process of isolation and purification because of dilution or decomposition [4]. Furthermore, it is difficult to obtain sufficient quantity of pure compounds for subsequent verification of biological activities. Hence, development of fast and robust screening methods for discovering active compounds from highly complex matrices is urgently required for pharmaceutical research. Over the past decades, several strategies were successfully adopted in the screening of complex mixture. The present review mainly provides an overview of the reported different strategies, which allow the simultaneous screening and characterization of active compounds from complex mixtures by integration of separation science, bioactivity screening and mass spectrometry identification, since 2000. In general, the applied strategies could be categorized into two modes, online post-column bioassay and pre-column affinity-based screening. Their principles and applications mainly for the discovery of active compounds including antioxidants, enzyme inhibitors and receptor ligands in herbs were discussed.

2. Discovery of natural antioxidants/free radical scavengers

2.1. Chromatographic separation methods

Antioxidants are known to be beneficial for preventing many physiological and pathological processes caused by free-radical reactions. Therefore, lots of methods have been developed for the screening antioxidants from natural products in the past few decades. Chromatographic separation with biochemical detection is the currently used methods for the discovery of antioxidants. Thin layer chromatography (TLC) [5], high performance liquid chromatography (HPLC) [6–8], high-speed counter-current chromatography (HSCCC) [9] and capillary electrophoresis (CE) [10,11] are available techniques, and HPLC is the most commonly used. Antioxidants in essential oil of *Angelica sinensis* [12] and Pu-erh tea [13] were rapidly screened and characterized using HPLC coupled with DAD-MS and ABTS-based assay in our previous study (for more applications of online methods, please refer to Refs. [6–8]). The sample extract was injected into HPLC system for separation and the eluent from DAD was split into two streams. One was introduced into MS for structure elucidation, and the other was used for antioxidant activity assay, where the continuous flow of reaction reagents solution like free radicals (e.g. $ABTS^{\bullet+}$ or $DPPH^{\bullet}$) was introduced into the reaction coil and interacted with the eluent. The mixed solution is monitored at the maximum wavelength of the free radicals, and a baseline is obtained if the eluent has no antioxidants. When the analyte with antioxidant activity was eluted, free radicals will be scavenged and the maximum wavelength is changed. Then a negative peak will be shown, which suggests the analyte has antioxidant activity. In this way, antioxidants were detected and easily identified based on HPLC chromatograms and MS data. However, it is worth noting that the initially observed effect of nature products may be the integrating action of multiple components with weak pharmacological activity. Therefore, some active components with trace amount and/or weak activity may be omitted when high resolution method such as HPLC is employed [14]. For example, the essential oil of *Curcuma wenyujin* has antioxidant activities *in vitro*, but no obvious antioxidant in the oil was found using HPLC separation coupled with ABTS-based assay [15]. Instead by low-resolution technique

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