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Review

Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach

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ABSTRACT

The phytochemical research based on ethnopharmacology is considered an effective approach in the discovery of novel chemicals entities with potential as drug leads. Plants/plant extracts/decoctions, used by folklore traditions for treating several diseases, represent a source of chemical entities but no information are available on their nature. Starting from this viewpoint, the aim of this review is to address natural-products chemists to the choice of the best methodologies, which include the combination of extraction/sample preparation tools and analytical techniques, for isolating and characterizing bioactive secondary metabolites from plants, as potential lead compounds in the drug discovery process. The work is distributed according to the different steps involved in the ethnopharmacological approach (extraction, sample preparation, biological screening, etc.), discussing the analytical techniques employed for the isolation and identification of compound/s responsible for the biological activity claimed in the traditional use (separation, spectroscopic, hyphenated techniques, etc.). Particular emphasis will be on herbal medicines applications and developments achieved from 2010 up to date.

Contents

1. Introduction

Plants, animals and micro-organisms represent a reservoir of natural products, the so called "natural sources deriving compounds". Particularly, the plant kingdom offers a variety of species still used as remedies for several diseases in many parts of the world such as Asia [1,2], Africa [3–6] and South America [7]. Even if, as reported by World Health Organization [8], traditional medicines represent the primary health care system for the 60% of the world's population, the plant species with possible biological activity remain largely unexplored [9]. As stated by Newman and Cragg in a recent review [10]: "natural product and/or natural product structures continued to play a highly significant role in the drug discovery and development process". Thus, biodiversity represents an unlimited source of novel chemicals entities (NCE) with potential as drug leads. These NCE are secondary metabolites, synthesized by plants as defence against herbivores and pathogens or attraction of pollinating agent, and can be grouped in three main chemical families: alkaloids, terpenoids and phenolic compounds.

A review from Kashani et al. [11] recently highlights the pharmacological properties of some well known secondary metabolites

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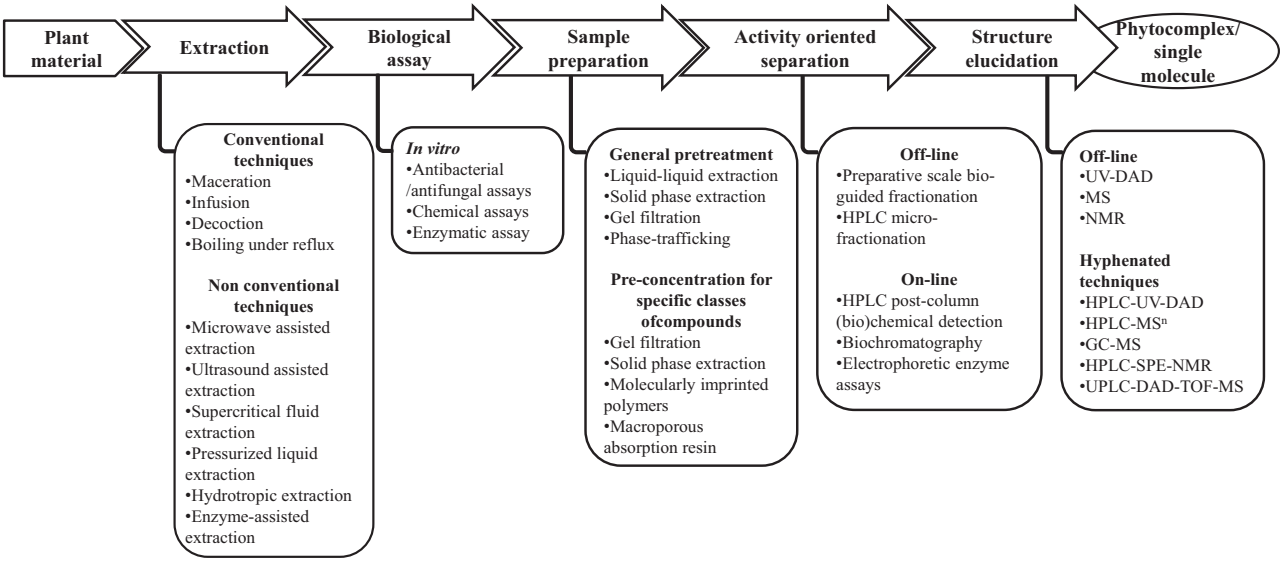

Fig. 1. Methodologies involved in the ethnopharmacology approach.

and many recent papers report the activity of new and/or less known alkaloids [12–14], terpenoids [15,16] and phenolic compounds [17–19] giving a direct evidence of the crucial role of natural products as potential sources of various modern pharmaceuticals. However, secondary metabolites are often present in low quantity in plant material and their extraction, purification and characterization still remain a great challenge in the drug discovery process. Several reviews have been recently published giving an overview on sample preparations [20–22] and characterization [23–25]. Although exhaustive in the treated field, these reviews basically deal with the chemotaxonomy-oriented approach: the plant species selected for screening are known to contain specific secondary metabolites (alkaloids, steroids, amino acids, etc.); thus, the choice of the more appropriate extraction methodology and the more suitable analytical technique is performed in order to achieve the best extraction/purification/separation of the desired secondary metabolite.

In the ethnopharmacological approach, the main requirement is the knowledge of the plant parts traditionally employed as remedies. The two main traditional medicines, Chinese and Ayurveda, have their ancient texts in the Chinese Materia Medica written by Shizhen at the time of the Ming Dynasty [26] and the ayurvedic Charaka Samhita written in Sanskrit probably around 400–200 before the common era, respectively. Both texts are now available as English version [27,28] and still used as references for herbal remedies [29–31]. Where tests are not available, the ethnobotanical survey is the only method for acquiring information on medicinal plants traditional use.

The phytochemical research based on ethnopharmacology is considered an effective approach in NCE discovery, however in this case no information are available on the nature of secondary metabolite; thus all the extraction/purification/separation processes are performed in order to "find and follow" the supposed pharmacological activity with the final aim to isolate and identify the bioactive compound/s.

Starting from the ethnopharmacological approach, the aim of this review is to address natural-products chemists to the choice of the best methodologies, which include the combination of extraction/sample preparation tools and analytical techniques, for isolating and characterizing bioactive NCE from plants, as potential lead compounds in the drug discovery process. A particular attention will be focused on herbal medicines applications and developments achieved from 2010 up to date.

An overview on the methodologies (extractive, biological, analytical) involved in the selected approach is shown in Fig. 1.

2. Extraction techniques and sample preparation

2.1. Extraction techniques

Extraction is the first step in the drug discovery process from plants. Several general procedures have been proposed for obtaining extracts representing a range of polarity [32] and/or enriched of the most common secondary metabolites such as alkaloids [33] and saponins [34].

Beyond the traditional solid–liquid extraction methodologies, such as maceration, infusion, decoction and boiling under reflux, a wide range of modern techniques have been introduced in the past decades. These include microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE), supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE).

In the MAE, for example, microwaves are combined with traditional solvent extraction; this non conventional heating system may enhance the penetration of solvent into the plant powder promoting the dissolution of the bioactive compounds, as described by Zhang et al. [35]. Similarly, in the UAE, the ultrasonic waves break the cell walls promoting the release of bioactive natural products into the solvent [36]. In a recent review Chang et al. [37] reported a comparison between MAE, UAE and conventional methodologies which highlights the advantages of MAE and UAE concerning extraction time (shorter) and extraction yield of bioactive components (higher). In this review the recent advancements in the development of MAE techniques also are reported. High pressure MAE (HPMAE), nitrogen protected MAE (NPMAE), vacuum MAE (VMAE), ultrasonic MAE (UMAE), solvent free MAE (SFMAE) and dynamic MAE (DMAE) are described and guidelines for selecting suitable techniques are well tabulated.

DMAE is particularly interesting since can be arranged for an on-line coupling with different chromatographic systems. Tong et al. developed an on-line method for the extraction and isolation of bioactive constituents from *Lyeicnotus pauciflorus* Maxim, a plant used in the traditional Chinese medicine for treating several diseases. Particularly, the coupling of DMAE with high-speed-counter-current chromatography allowed a continuous isolation of the major active constituent nevadensin, in higher yield and