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Bioassay guided fractionation-an emerging technique influence the isolation, identification and characterization of lead phytomolecules

Neelesh Malviya*1, Sapna Malviya 2

* 1 Smriti College of Pharmaceutical Education, Indore

2 Modern Institute of Pharmaceutical Sciences (MIPS), Indore

ABSTRACT

Natural products have thus played an important role in drug discovery in the past and promise to provide still more drugs in the future. The search for a new drug from nature is based on a biological and ecological rationale. Natural products have provided many effective drugs. These include older drugs such as quinine and morphine and newer drugs such as paclitaxel, camptothecin, etoposide, mevastatin, and artemisinin. The discovery of novel drugs from nature is also important because many isolated molecules are quite complex, and would not be obtained by a simple synthetic approach. Most bioactive compounds of natural origin are secondary metabolites, i.e. species-specific chemical agents that can be grouped into various categories. A typical protocol to isolate a pure chemical agent from natural origin is bioassay-guided fractionation, meaning step-by-step separation of extracted components based on differences in their physico-chemical properties, and assessing the biological activity, followed by next round of separation and assaying.

Key words: Drug discovery, Natural products, bioassay-guided fractionation

*Correspondence to Author:

Dr. Neelesh Malviya

Associate Professor, Smriti College of Pharmaceutical Education, Indore. Mob: 9826319555, Email: nil30oct@gmail.com

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INTRODUCTION

Traditional medicine is the knowledge, skills and practice of holistic health care, recognized and accepted for its role in the maintenance of health and the treatment of diseases. It is based on indigenous theories, beliefs and experiences that are handed down from generation to generation. Traditional medicine is practised in many countries it has always been part of the cultural and religious life of Indian people [1]. Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace. Today, more than 80% population of the developing countries depends on plants for their medical needs [2]. In addition, the use of ethno medical information has contributed to health care worldwide through the isolation of bioactive compounds for direct use in medicine. The use of medicinal plants in the form of crude extracts presents several difficulties. The amount of the bioactive compound(s) from plants may vary with both the locality and the season in which they are collected. Also, bioactive molecules of many plants are powerful poisons when taken in excess, and if the plant extract contains a lower content of bioactive compound(s) than usual, suboptimal dosage may not be effective. Medicinal properties of many plants are also rapidly lost on storage. Furthermore, crude extracts from many medicinal plants may contain, in addition to the bioactive molecules, other constituents which have harmful effects. It is therefore important to isolate and identify the bioactive molecules from plant extracts. Structural modification of isolated and identified bioactive compounds from plant extracts may allow an improvement in the efficacy and moderation of side effects. Medicinal plants have become the focus of intense study recently in terms of discovering new drugs and as to whether their traditional uses are supported by pharmacological effects [3]. The R&D thrust in the pharmaceutical sector is focused on development of new drugs, innovative/indigenous processes for known drugs and development of plant-based drugs through investigation of leads from the traditional systems of medicine [4]. Traditional herbs represent an extraordinary reservoir of active ingredients which are still present in about 25% of all prescriptions of modern "Western" medicine [5]. Phytochemicals have evolved from traditional medicinal plants to modern sci-

entific medicine, giving support to the empirical knowledge of "alternative" healing [6]. Globally, at least 119 compounds derived from 90 plant species can be considered as important drugs. 74% of these substances were found by the chemical studies through the isolation of the bioactive compounds from plants used in traditional medicine [7]. In the early nineteenth century, isolating the active compounds from extracts was involved for the use of medicinal plants. Up to the 30's of last century, a series of natural products isolated from plants became clinical agents and a number of that is still in use today. More recently, isolation and characterization of pharmacologically active compounds from medicinal plants for drug discovery continue and become more efficient by applying the new technologies. Nowadays there is a renewed interest in investigating plants for medically useful compounds, with some of the leading pharmaceutical and research institutions involved in this search. More than 50% of the 25 best-selling drugs worldwide were related directly to natural products [8]. A deeper understanding of phytochemistry, pharmacognosy and ethnopharmacology should therefore be encouraged to support the production of new and safe pharmacologically active compounds with minimal undesired toxic effects. Drug discovery from medicinal plants led to the isolation of early drugs, such as quinine from *Cinchona* bark, morphine and codeine from the opium poppy, digoxin from *Digitalis* leaves and podophyllotoxin from the rhizome of *Podophyllum* some of which are still in clinical use and other serve as lead compounds for the synthesis of new biologically accepted compounds [9].

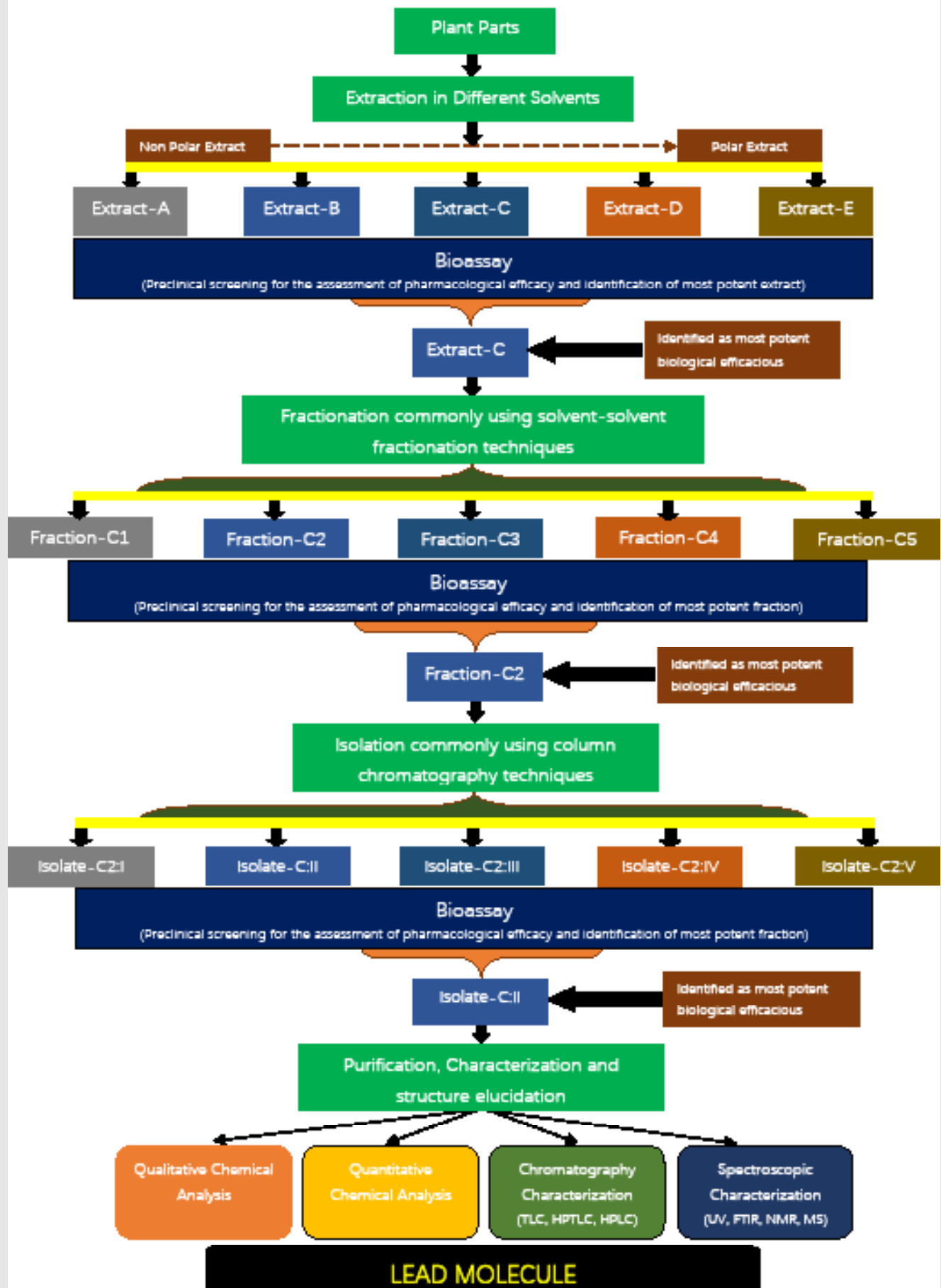
BIOASSAY GUIDED FRACTIONATION-AN EMERGING TECHNIQUE:

Bioassay guided fractionation of plant extracts linked to chromatographic separation techniques can lead to isolation of biological active molecules (Figure No. 1). As a result of the recent interest in the plant kingdom as a potential source of new drugs, strategies for the fractionation of plant extracts based on biological activity rather than on a particular class of compound have been developed. The chemical examination follows after the isolation of the active fraction [8]. The search for a new drug from nature is based on a biological and ecological rationale. Natural products have provided many effective drugs.

Table 1: Bioassay-guided fractionation technique based isolated compounds

Plant name	Isolated compounds	Therapeutic efficacy	Reference
<i>Hypericum</i> species (Hypericaceae)	Benzopyrans and phloroglucinol	Antimicrobial	[11]
<i>Kaempferia galanga</i> L. (Zingiberaceae)	Ethyl cinnamate and Ethyl p-methoxycinnamic	Vasorelaxant	[12]
<i>Melissa officinalis</i> L. (Lamiaceae)	Rosmarinic acid, ursolic acid and oleanolic acid	Potent inhibitor of GABA-T	[13]
<i>Centaurea arenaria</i> M.B. ex Willd. (Compositae)	Eupatilin, eupatorin, 3'-methyleupatorin, apigenin, isokaempferid, arctigenin, arctiin, matairesinol, moschamine, cis-moschamine, β -amyryn, and β -sitosterin- β -D-glycopyranoside	Antiproliferative	[14]
<i>Anthemis ruthenica</i> M. (Asteraceae)	Eudesmanolide sesquiterpene, sivasinolide 6-O-angelate and centaureidin	Cytotoxic	[15]
<i>Sorbus decora</i> Sarg. (Rosaceae)	Pentacyclic triterpenes 23,28-dihydroxyursan-12-ene-3 β -caffeate, 23,28-dihydroxylupan-20(29)-ene-3 β -caffeate, and 3 β ,23,28-trihydroxy-12-ursene	Antidiabetic	[16]
<i>Glycyrrhiza uralensis</i> Fisch. (Fabaceae)	Glycyrrhisoflavone	α -glucosidase inhibitory	[17]
<i>Vaccinium arctostaphylos</i> L. (Ericaceae)	Malvidin-3-O-beta-glucoside	α -amylase inhibitor	[18]
<i>Tagetes patula</i> L. (Asteraceae).	Methyl protocatechuate, patuletin and patulitrin	Antioxidant with analgesic properties	[19]
<i>Tithonia diversifolia</i> Hemsl. (Asteraceae)	Tagitinin C	Anti-ulcer	[20]
<i>Cassia bakeriana</i> Craib. (Fabaceae)	Cassic acid or rhein	Antimicrobial and cytotoxic activities	[21]

Figure 1: Bioassay-guided fractionation protocol for the isolation of biological active compounds



These include older drugs such as quinine and morphine and newer drugs such as paclitaxel, camptothecin, etoposide, mevastatin, and artemisinin. Natural products have thus played an important role in drug discovery in the past and promise to provide still more drugs in the future. The discovery of novel drugs from nature is also important because many isolated molecules are quite complex, and would not be obtained by a simple synthetic approach. Most bioactive compounds of natural origin are secondary metabolites, i.e. species-specific chemical agents that can be grouped into various categories. A typical protocol to isolate a pure chemical agent from natural origin is bioassay-guided fractionation, meaning step-by-step separation of extracted components based on differences in their physicochemical properties, and assessing the biological activity, followed by next round of separation and assaying [10]. Therapeutically active phytoconstituents isolated from higher plants have been providing novel, clinically active drugs. The key to the success of discovering naturally occurring therapeutic agents rests on bioassay-guided fractionation and purification procedures. Bioassay-guided fractionation is a procedure, whereby extract is chromatographically fractionated and refractionated until a pure biologically active compound is isolated. Each fraction produced during the fractionation process is evaluated in a bioassay system and only active fractions are fractionated.

In last decade by using bioassay-guided fractionation technique various effective compounds are isolated successfully, some of them are summarized in Table 1.1.

CONCLUSION

Development of new drug is a complex, time-consuming, and expensive process. The time taken from discovery of a new drug to its reaching the clinic is approximately 12 years, involving more than 1 billion US\$ of investments in today's context. Essentially, the new drug discovery involves the identification of new chemical entities, having the required characteristic of drug ability and medicinal chemistry [22]. The sources of many of the new drugs and active ingredients of medicines are derived from natural products. Bioassay-guided fractionation method is commonly employed in drug discovery research due to its

effectiveness to directly link the analyzed extract and targeted compounds using fractionation procedure that followed with certain biological activity.

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