



Natural Products' Extraction and Isolation-Between Conventional and Modern Techniques

Usama Ramadan Abdelmohsen^{1,2*}, Ahmed M. Sayed^{3,4} and Abeer H. Elmaidomy⁵

¹Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt, ²Department of Pharmacognosy, Faculty of Pharmacy, Deraya University, New Minia, Egypt, ³Department of Pharmacognosy, Faculty of Pharmacy, Nahda University, Beni-Suef, Egypt, ⁴Department of Pharmacognosy, Faculty of Pharmacy, Almaaqaq University, Basra, Iraq, ⁵Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt

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Natural products (NPs) are significant for drug development. They appear in consistently low amounts in natural medicines (Sturm and Seger, 2012). Now, the advancement of adequate and selective techniques for the extraction and isolation of those bioactive NPs is vital (Rosén et al., 2009). Despite serious increases in extraction-separation procedures, the isolation of NPs from marine creatures, microorganisms, or plants is still a challenging task (Rosén et al., 2009). Hybrid techniques like LC-MS or LC-NMR meant on-line structure elucidation remained possible and showed impressive illustrations of NPs' recognition without early isolation. However, in many examples, the requirement to show the isolated NPs is still needed (Sturm and Seger, 2012). Detailed chemical designs involving stereochemistry of recent NPs often require separated, highly purified, isolated compounds (Sturm and Seger, 2012). Furthermore, assessing biological activity *in vitro* and/or *in vivo* must be determined after purification to remove conflict with turbulent compounds (Sturm and Seger, 2012). Writing rules for the quality management of medicinal herbal plants principally hinges on compound separation with significant purity (Sturm and Seger, 2012). The classic isolation system of NPs involves recognition, selection, and formation of the biological equipment. Descent with different solvents from limited to stronger polarity.

Bioassay-guided isolation schemes associating data on the chemical surveys of crude extracts and portions with their enterprise data for *in vitro* bioassays observed in micro-scale automatically shortened the duration for target detection (Molyneux et al., 2007). Fundamentally, it has proven to be a distinct step to pick up from a natural source to an intense isolate. However, some crucial steps must be recognized, such as correct natural source recognition, awareness of conversions during preparation and/or extraction, or previously recognized compounds' de-replication at the fractionation scheme basic stage (Molyneux et al., 2007).

Most research intending to isolate substantial amounts of pure isolated compounds still employs the wide use of liquid chromatographic techniques as MPLC, VLC, and HPLC (van Beek et al., 2009). Taking advantage of enhanced separation efficiencies allows the involvement of slight particle diameter and distinct selectivity. Solid-phase extraction, basically set up as a purification technique before GC or HPLC analysis, is accepted as a technique for rapid fractionation of raw extracts or for trapping pure isolates that are eluted after HPLC separation brought to capillary NMR test for *de novo* design elucidation (van Beek et al., 2009). The main aim of this article is to summarize the various extraction and isolation techniques of NPs. It chiefly focused on the analytical procedures that incorporate the extraction methods and isolation of NPs between conventional and modern techniques.

Figure 1 summarizes the different isolation methods that are currently applied to natural products during the drug discovery pipeline.

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*Correspondence:

Usama Ramadan Abdelmohsen
usama.ramadan@mu.edu.eg

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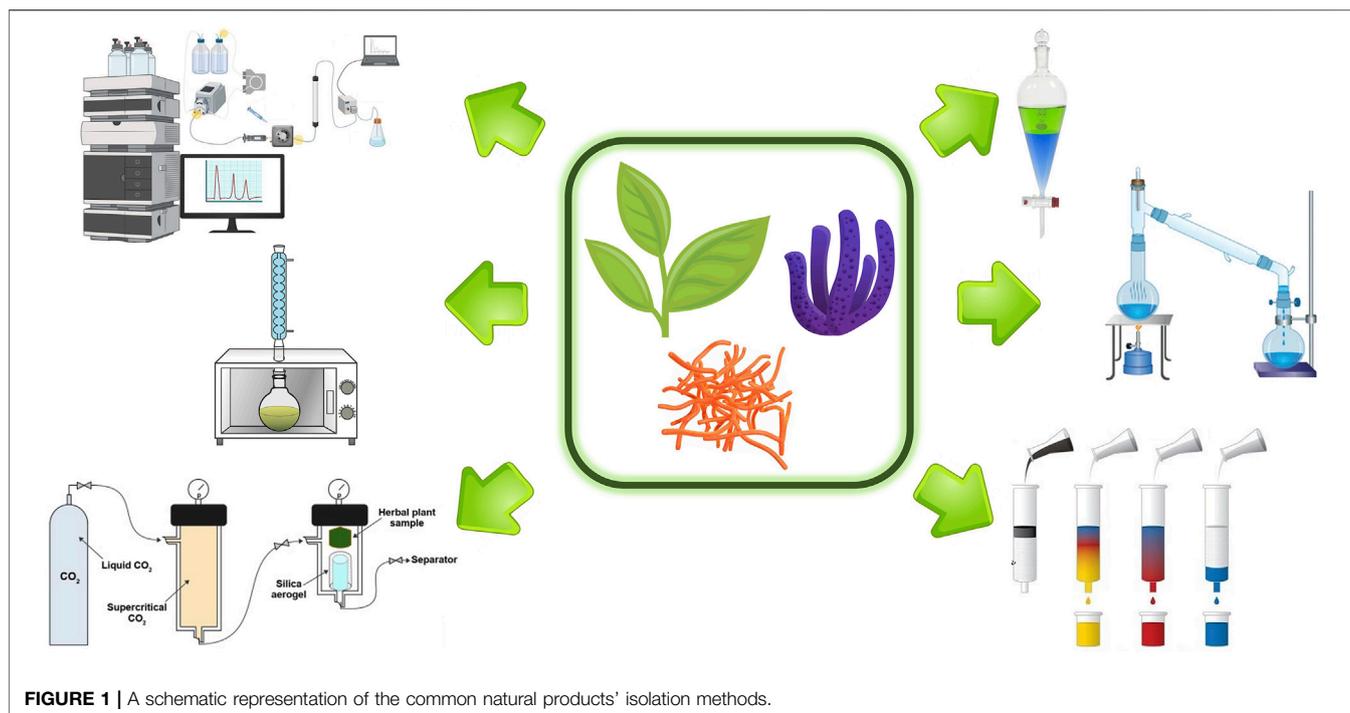
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EXTRACTION METHODS

Extracting NPs from their natural source involves many issues. These issues include the stability and polarity of the solvent and extractives, the volatility, toxicity, purity, and viscosity of the extractive solvent (Maltese et al., 2009), artefact development possibility during the process of extraction, and the bulk material output to be extracted (Maltese et al., 2009).

Classical Solvent Extraction

Most isolation operations still employ basic extraction programs with water, organic solvents of various polarities, and their mixes (Bucar et al., 2013). These processes involve infusion, decoction, maceration, percolation, Soxhlet, and reflux extraction. Infusion is achieved by suspending the material into solvent over a short period. In decoction, the material is boiled to dissolve its chemicals. Consequently, this is suitable only for extracting water-soluble and heat-stable constituents. Maceration is brought out at the chamber with heat (also known as digestion), or without it in a closed container by immersing the material in the extracted solvent with or without consequent stirring (Bucar et al., 2013). It benefits under moderate descending circumstances, but suffers from excessive solvent usage, lengthy extraction phases, and poor extraction crops. Extraction output is enhanced by percolation, in which the pre-wet material is loaded in a package which provides the regularly adjusted elimination of the excerpt *via* a pipe at the basement and continuous fresh solvent from top; percolation suffers from excessive solvent utilization and tedious extraction stages. Another attractive extraction method is using Soxhlet apparatus, as it diminishes solvent utilization. It effectively recycles a limited volume of solvent to dissolve a greater

extent of material. Reflux is an extraction process carried out at a stable heat with repeatable solvent evaporation and condensation for a particular time without the loss of solvent. Soxhlet and reflux are efficient, simple to handle, and cost effective. However, Thermo-labile isolates are impaired during the eradication process using heat (Bucar et al., 2013).

Ultrasound Assisted Extraction

In the UAE, the material is generally put in a glass vessel, soaked in the extraction solvent, and placed in an ultrasonic bath. It reduces the time of extraction and promotes the yields owing to automatic stress, which leads to cavitation with cellular disruption (Bucar et al., 2013). UAE can also be used with mixtures of immiscible solvents, such as hexane with methanol/water. But the process creates heat, so Thermo-labile isolates are impaired during the eradication process (Bucar et al., 2013).

Microwave Assisted Extraction

Today, extraction using either diffused microwaves in sealed techniques or focused microwaves in open procedures are accepted techniques. MAE has been modified into various forms, such as nitrogen-protected microwave-assisted extraction (NPMAE), vacuum microwave-assisted extraction (VMAE), dynamic microwave-assisted extraction (DMAE), or ultrasonic microwave-assisted extraction (UMAE) (Bucar et al., 2013).

Ionic Liquids Assisted Extraction

In recent periods, utilization of ILs for MAE, UAE, or basic extraction has been receiving increased attention. ILs are organic salts which, when in the liquid state, interact with an organic/

inorganic anion and an organic cation. ILs can dissolve a large area of polar/non-polar NPs, have a low vapor pressure, exhibit a great thermal establishment, and have low combustibility. Another aspect of ILs that is still inadequately studied is their biodegradability and their impact on the habitat if applied at industry; both require further study (Bucar et al., 2013).

Accelerated Solvent Extraction

In correlation to most alternative extraction procedures which require an added step to split the standing non-dissolved matter from the liquid excerpt, on-line filtration through the automated descent process of ASE is introduced. The technique is used with solid/semisolid samples (1–100 g scale), and can handle solvents at raised heat and pressure (Bucar et al., 2013).

Supercritical Fluid Extraction

Changing organic extraction solvents with technologies for extraction—which less harmful to the environment—can be viewed as an encouraging force to increase demand of supercritical fluid extraction. An outline of the procedure, with extraction contracts and operations in NPs' extraction, is given by Nahar and Sarker 2012 (Nahar and Sarker, 2012). Application of organic solvent modifiers in SFE extends its solvating efficiencies to medium-polar, and polar NPs, and expands the scope of NPs classes available to SFE. Using this technique, ecological complications related to organic solvent descents are reduced to a limited extent (Bucar et al., 2013).

Solid Phase Extraction

Extraction, which can adsorb analyte or undesirable contaminations in a solid phase, achieved a prominent character in NPs extracts purification, owing to its assimilation into computerized specimen formation and isolation processes. Most applications employ SPE, which uses a broad field of stationary phases with differing chemistry as reversed-phase material, silica gel, ion-exchange resins, and HILIC stationary phases in pre-loaded plastic or glass columns.

Distillation

Volatiles, as essential oils, have till now been gained through distillation procedures, although working at raised temperatures can result in chemical transformations. New improvements in distillation methodology have resulted in microwave steam distillation, which uses microwaves to raise the interruption of glands and cells at the same time for steam crossing through the plant cell material and obtaining the essential oil (Bucar et al., 2013).

ISOLATION OF NATURAL PRODUCTS USING DIFFERENT CHROMATOGRAPHY TECHNIQUES

A broad range of chromatographic methods is applicable for better fractionation and finished NPs' purification. The choice generally revolves around the purity stage of the extract or fraction, and the decisive purpose of the isolated NPs.

Preparative Planar Chromatography

Due to its low cost and easy usage in isolation of NPs, PPC has been an often-employed procedure, although the number of applications is less compared to column chromatography (Marston, 2011). An interesting aspect of PPC is the broad field of chemical disclosure techniques characteristic for NPs, which can be achieved on a limited area of the plate, leaving most compounds consistent and convenient for isolation. To reduce the uncontrolled flow rates for the classical TLC mobile phase, forced-flow procedures such as over-pressured layer chromatography or centrifugal planar chromatography have been established, permitting elution and on-line disclosure of compounds (Marston, 2011).

Column Chromatographic

Vacuum liquid chromatography (VLC) (Reid and Sarker, 2012), flash chromatography (FC) (Reid and Sarker, 2012), hydrophilic interaction chromatography (HILIC) (Reid and Sarker, 2012), low-pressure liquid chromatography (LPLC) (Reid and Sarker, 2012), medium-pressure liquid chromatography (MPLC) (Cheng et al., 2010), and high-performance (high-pressure) liquid chromatography (HPLC) (Alpert, 1990) are used.

Chiral Chromatographic

After chiral compounds' isolation, generally a process to detect absolute configuration is required. For enantio-separation at an analytical scale, GC, HPLC, SFC, or CE have universally been applied. However, HPLC is used most frequently. This separation procedure provides insulating enantiomers directly with chiral stationary or mobile-phase, or indirectly with chiral derivatization agents' additives (Dalgliesh, 1952).

Preparative Gas Chromatography

PGC is an interesting choice for the isolation of volatile oils. Mostly, loaded columns with greater sample capacity but lower peak resolution are established. However, there have been an expanding number of outstanding operations of thick-phase film full-bore capillaries with capillary GC apparatus during the last years (Zuo et al., 2012).

CONCLUSION

Recently, several significant improvements have been made in the NPs isolation field. A rising number of techniques have been built up by chromatographic hyphenation and spectroscopic means with the objective of elucidating structures of the known compounds without the need for isolation. MAE and UAE strategies are accepted as systems that increase extraction potency and reduce the descent stage. ILs are a further impending field in the natural products field and could improve the interest compounds. SPE broadened its utilization towards fractionation procedures like VLC. But one extremely interesting outcome in SPE is the selective isolation of objective compounds by molecularly engraved stationary phases. Chiral separations are also applied at preparations scale, taking the chiral character of many NPs into interest. HILIC is currently experiencing a significant expansion in applications for NPs isolation, producing an added mechanism of separation related to

normal and reversed-phase chromatography. Although pure compounds' isolation from raw matrices is still challenging and we are far from one-step isolation methods, the analysis of extremely selective processes of extraction to fractionation and purification will improve the collection of a final purified compound.

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