PharmacoLearn

MPG103T Phytochemistry, M.Pharm Semester 1

Unit 3: Extraction and Phytochemical Studies

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3.1 Selection of method and choice of solvent

Extraction is the transfer of the wanted constituents from the plant material into a solvent, leaving as much of the unwanted matrix behind as the purpose allows. The syllabus puts selection first, of method and of solvent, because almost every later success or failure is decided there. Solvent choice follows like dissolves like, made precise by polarity: non polar solvents such as hexane and petroleum ether take fixed oils, waxes and the least polar terpenoids; medium polarity solvents such as chloroform, dichloromethane and ethyl acetate take free alkaloid bases, aglycones and many terpenoids; polar solvents, ethanol, methanol and water and their mixtures, take glycosides, saponins, tannins, alkaloid salts and sugars. Ethanol and its aqueous mixtures are the industry's workhorse solvents: wide polarity range, comparatively safe, acceptable in products for human use; methanol is the laboratory's wider net, used analytically. Four further factors complete the choice: stability (a thermolabile constituent forbids a boiling solvent; an easily hydrolysed one forbids water and acid or alkali unless they are the point); selectivity (the best solvent extracts the wanted constituent with the least companion material, not the most material overall); safety and legality (flammability, toxicity, residue limits); and purpose (assay, isolation and manufacture extract differently). Method choice follows the material: particle size and hardness, the constituent's location, the scale, and the stability limits already set.

3.2 The commonly used methods of extraction

The classical methods remain in daily use because each is the right tool for one kind of material. **Maceration** soaks the powdered drug in solvent in a closed vessel for days with occasional shaking, then strains and presses the marc; it suits thermolabile constituents and small scale work, at the cost of time and incomplete extraction. **Percolation** packs the drug in a percolator and lets solvent flow slowly through the bed; it is the pharmacopoeial method for tinctures and many extracts, more complete than maceration and run cold where needed. **Infusion and decoction** are the aqueous traditional forms, a short hot steep for delicate material and boiling for hard material such as roots and barks, suited to water soluble constituents and immediate use. **Digestion** is maceration under gentle heat. **Reflux extraction** boils the drug with solvent under a condenser, fast and complete for heat stable constituents. **Soxhlet extraction** is the laboratory's exhaustive continuous method: solvent vapour condenses onto the drug in a thimble, the chamber siphons back, and the drug is washed repeatedly with effectively fresh hot solvent while the extract concentrates in the flask; its standing limitation is that the extract sits at boiling point for hours, excluding thermolabile constituents. **Distillation methods** serve the volatile oils: hydrodistillation in the Clevenger type apparatus for assay, steam distillation at production scale. The figure below compares the working set.

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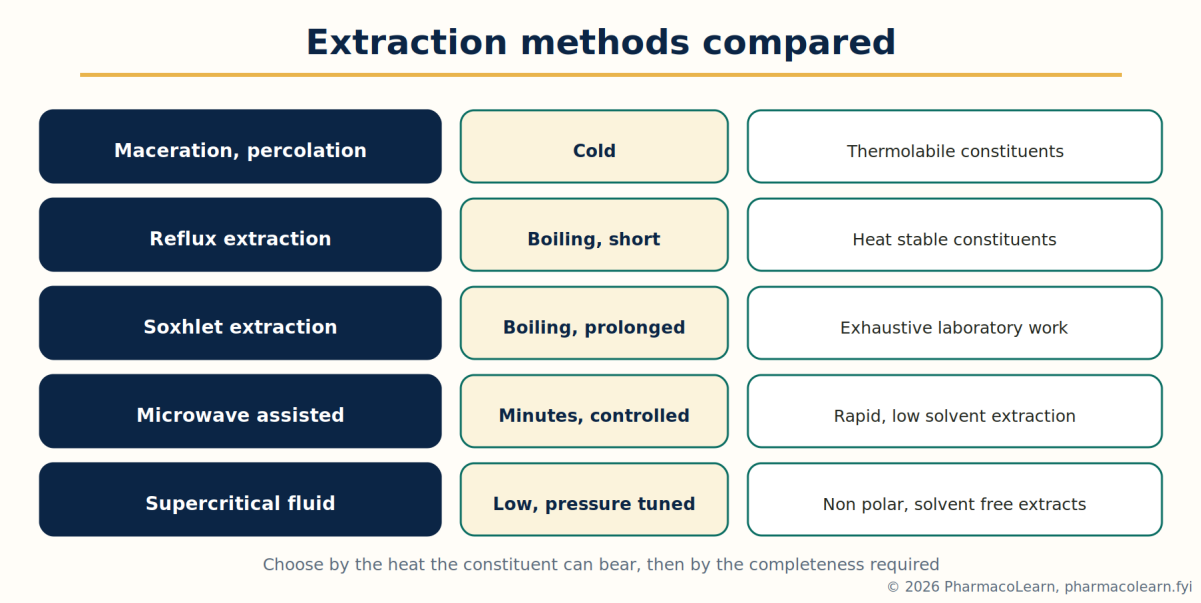

Figure: The extraction methods compared. Heat and completeness rise together up the classical ladder; the modern methods of Section 3.4 break that trade by adding energy or pressure instead of time.

3.3 Successive and exhaustive extraction

Two syllabus terms organise extraction strategy and are often confused. **Exhaustive extraction** is extraction continued until the drug gives up effectively all of the extractable constituent; the last portion of solvent shows no further constituent by the chosen check. Soxhlet extraction is exhaustive by design, and percolation is run to exhaustion for a complete extract. **Successive extraction** is a strategy, not a degree of completeness: the same marc is extracted in turn with solvents of increasing polarity, classically petroleum ether, then chloroform or ether, then alcohol, then water, each extract collected separately. It is therefore the first fractionation as well as an extraction: the non polar extract carries oils, waxes and free terpenoids, the medium extracts aglycones and free bases, and the polar extracts glycosides, saponins and tannins, so the scheme recovers the constituents already sorted by class for phytochemical screening. Its discipline is order and completeness: each solvent is run to exhaustion before the next begins.

3.4 Recent advances in extraction: microwave assisted extraction and the modern methods

Recent advances share one aim: to get the constituent out faster, with less solvent and less damage, by putting energy or pressure where the plant structure resists. **Microwave assisted extraction (MAE)** is the method the syllabus names. Microwaves heat the water inside the plant cells directly, because the field acts on polar molecules; the heated water builds pressure, the cell walls rupture, and the constituents are released into the surrounding solvent, which may stay comparatively cool. Extraction times fall from hours to minutes and solvent volumes fall, with the cautions that the solvent must interact with the field or the material must carry moisture, and that temperature must be controlled for thermolabile compounds. Beside MAE the modern set should be known in outline. **Ultrasound assisted extraction** uses cavitation, the collapse of microscopic bubbles, to batter cell walls; it is simple and cold running. **Enzyme assisted extraction** uses cellulase and pectinase type preparations to digest the wall itself. **Pressurised liquid extraction** keeps a solvent liquid above its boiling point under pressure, gaining hot solvent speed in a fraction of Soxhlet's exposure. **Supercritical fluid extraction**, treated in Section 3.6, replaces the liquid solvent with a dense gas whose power is tuned by pressure. The comparison table fixes the set.

Method	Driving force	Heat exposure	Best suited to
Maceration	Diffusion into standing solvent	None (cold)	Thermolabile constituents, small scale
Percolation	Solvent flow through a packed bed	None to low	Tinctures and pharmacopoeial extracts
Soxhlet	Repeated washing with condensing solvent	High and prolonged	Exhaustive laboratory extraction of stable constituents
Microwave assisted	Intracellular water heated, cells rupture	Short, controlled	Rapid marker extraction, reduced solvent work
Ultrasound assisted	Cavitation disrupts walls	Low	Fast laboratory and pilot extraction
Supercritical fluid	Tunable dense gas solvent	Low	Non polar constituents, clean extracts, volatile oils and decaffeination type work

3.5 Methods of fractionation, and the isolation scheme for a crude extract

Extraction gives a mixture; fractionation begins the sorting. The standard methods, in their usual order, are these. **Solvent partitioning** distributes the crude extract between immiscible solvents, classically from water or aqueous alcohol into hexane, chloroform or dichloromethane, ethyl acetate and n-butanol in turn: each layer is a fraction sorted by polarity, the saponins preferring butanol and the aglycones ethyl acetate. **Acid base fractionation** washes an alkaloid bearing extract between acid and alkali, as in Unit 1, separating bases from neutrals and acids. **Precipitation and crystallisation** drop a constituent or an impurity out of a concentrate by solvent change, cooling or reagent addition, lead acetate precipitation of tannins being the classical clean up. **Adsorption clean up** strips chlorophyll, tannins or pigments on a short adsorbent or polyamide bed. **Size based fractionation**, dialysis, membrane filtration and gel filtration, sorts by molecular size. The full scheme for working a crude extract to a pure constituent is the daily algorithm of the phytochemistry laboratory.

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Crude extract (defined solvent, defined drug to solvent ratio)
        |
Defat and clean up where needed (hexane wash, pigment removal)
        |
Solvent partitioning: hexane, chloroform, ethyl acetate, butanol, water
        |
Bioassay and marker assay of every fraction
        |
Active or marker rich fraction taken forward
        |
Column chromatography, flash or countercurrent separation
        |
Preparative HPLC for final separation of close compounds
        |
Crystallisation; purity by single peak and constant constants
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Pure phytoconstituent, ready for structure elucidation
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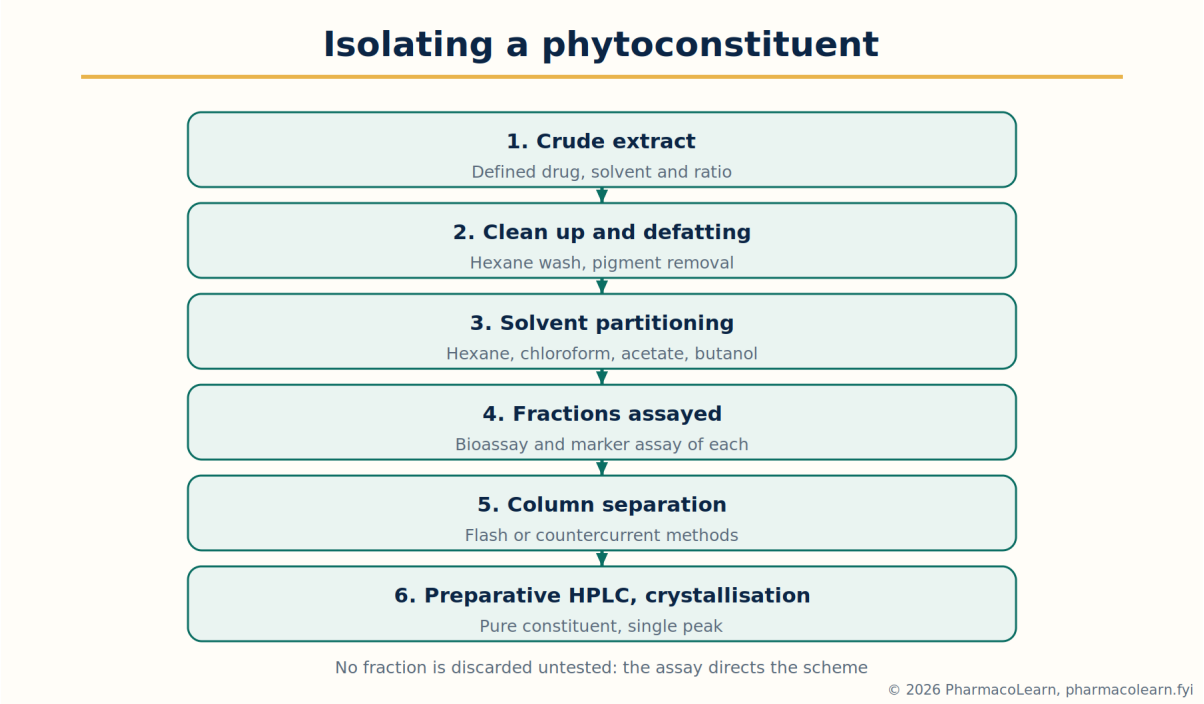

Figure: The isolation scheme for a crude extract. Every fraction is assayed before any is discarded; the scheme is Unit 3 drawn as one page.

3.6 Separation by CCCET and SCFE techniques

The syllabus sentence for this section reads: separation of phytoconstituents by latest CCCET and SCFE techniques including preparative HPLC and flash column chromatography. The abbreviation CCCET is printed without expansion, and these notes do not invent one for it, as the verification record flags. What can be taught honestly is the technique family its context points to, the countercurrent family, in which two immiscible liquids do the separating and there is no solid stationary phase, together with SCFE, read in context as supercritical fluid extraction. Both are taught on that openly stated basis.

Countercurrent separation works by partition alone. One liquid phase is held stationary and the other pumped through it; each constituent distributes itself between the phases by its partition coefficient and travels at its own rate, liquid liquid extraction repeated hundreds of times in one instrument. Its instrument form is countercurrent chromatography, including its high speed planetary centrifuge forms, in which the stationary liquid is retained in a coiled column by centrifugal force. Its virtues are characteristic: no solid adsorbent means no irreversible adsorption, so delicate polar compounds, saponins, glycosides and tannins, are recovered fully; capacity is large; and the solvent system is designed from test tube partition experiments by the logic of Section 3.5. Craig countercurrent distribution is the historical form of the same idea.

Supercritical fluid extraction and separation (SCFE) uses a fluid above its critical temperature and pressure, in practice almost always carbon dioxide, whose solvent strength is tuned by changing the density: low density carbon dioxide takes the most non polar constituents, higher density takes more, and a polar co solvent such as ethanol extends the range to medium polarity. On releasing the pressure the carbon dioxide leaves as a gas, so the extract is solvent free by construction and the working temperature is low, suiting thermolabile and volatile constituents. Decaffeination, hops and spice extraction and volatile oil work are the standard industrial examples. Its limitation mirrors its virtue: very polar constituents such as most glycosides dissolve poorly even with co solvents, so SCFE complements rather than replaces solvent extraction.

3.7 Preparative HPLC and flash column chromatography

The two named chromatographic techniques divide the final separation work by scale and resolving power. **Flash column chromatography** is rapid medium pressure column chromatography on silica gel of defined particle size, the solvent driven by air or nitrogen pressure rather than gravity. It is the workhorse purification step: gram scale loads, runs in tens of minutes, fractions monitored by thin layer chromatography, and resolving power well beyond a gravity column because the finer, uniform packing sharpens the bands. Its method is chosen from TLC first: the solvent system placing the target band at a suitable retention on a plate is transferred to the column, commonly as a gradient, and that plate to column discipline is the practical skill taught. **Preparative HPLC** is high performance liquid chromatography run for collection: larger columns and loads and a fraction collector in place of a purely recording detector. It is the final tool, used where flash chromatography cannot resolve the mixture, for close alkaloid pairs, taxane relatives, the dimeric vinca alkaloids and the last purification before structure proof. Its cost in solvent and time is why it stands last in the scheme of Section 3.5, applied only to fractions already cleaned and enriched. Separation is staged: cheap bulk methods first, resolving power spent only where it is needed.

3.8 Unit 3 in one view

Choose the solvent by the constituent's polarity and stability and the method by the heat it can bear; extract successively to sort by class or exhaustively to recover in full; and use the modern methods, microwave assisted extraction above all, where speed and solvent saving matter. Fractionate by partitioning, acid base behaviour, precipitation and size, following activity and markers at every step. Separate finally by the staged ladder: countercurrent methods where a solid phase would lose the compound, supercritical fluid extraction for a clean non polar extract, flash chromatography for working purification and preparative HPLC for the separations nothing else can finish.

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