Plant Extraction Protocols

Maryam Hameed Alwan

Department of oral diagnosis
College of Dentistry
University of Baghdad

TODAY'S DISCUSSION

- Introduction
- Conventional Methods for Plant Extraction
- Non-Conventional Methods for Plant Extraction
- Identification and Characterization
- Conclusion

INTRODUCTION

- World Health Organization (WHO) reported that more than 80% of the global population relies on traditional **medicine** for their primary healthcare needs.

- Even in developed countries, plant-based traditional medicines are often termed **complementary or alternative medicine** (CAM), and their use has increased steadily. For example, in the USA alone, the total estimated that herbal sales for 2005 were $4.4 billion, a significant increase from $2.5 billion in 1995

Contd…

- A sum of 20,000 plants has been identified in 91 countries that contain medicinal properties.

- **Medicinal plants** are now more focused than ever because they have the capability of producing many benefits to society especially for medicinal uses. The medicinal power of these plants is due to their phytochemicals which can cause definite physiological actions on the human body.

- Phytochemicals are natural compounds which occur in plants such as medicinal plants, vegetables, and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases

- These bioactive compounds are found in very small amounts in plants and can be used to improve good health.
- polyphenols, alkaloids, terpenes, and saponins are common bioactive compounds.
- These pure compounds or **standardized** extracts are reported to have many beneficial activities such as antimicrobial, anticancer, analgesic and wound healing activity.
- The use of these bioactive compounds in the pharmaceutical, food product industry signifies the need to have a standard method to extract them from the plants.

Contd...

- To utilize the biologically active compounds from the plant, the compounds need to be extracted, screened, isolated and characterized.

- Then the **bioactive compound** could be evaluated toxicologically and clinically for any natural supplement development.

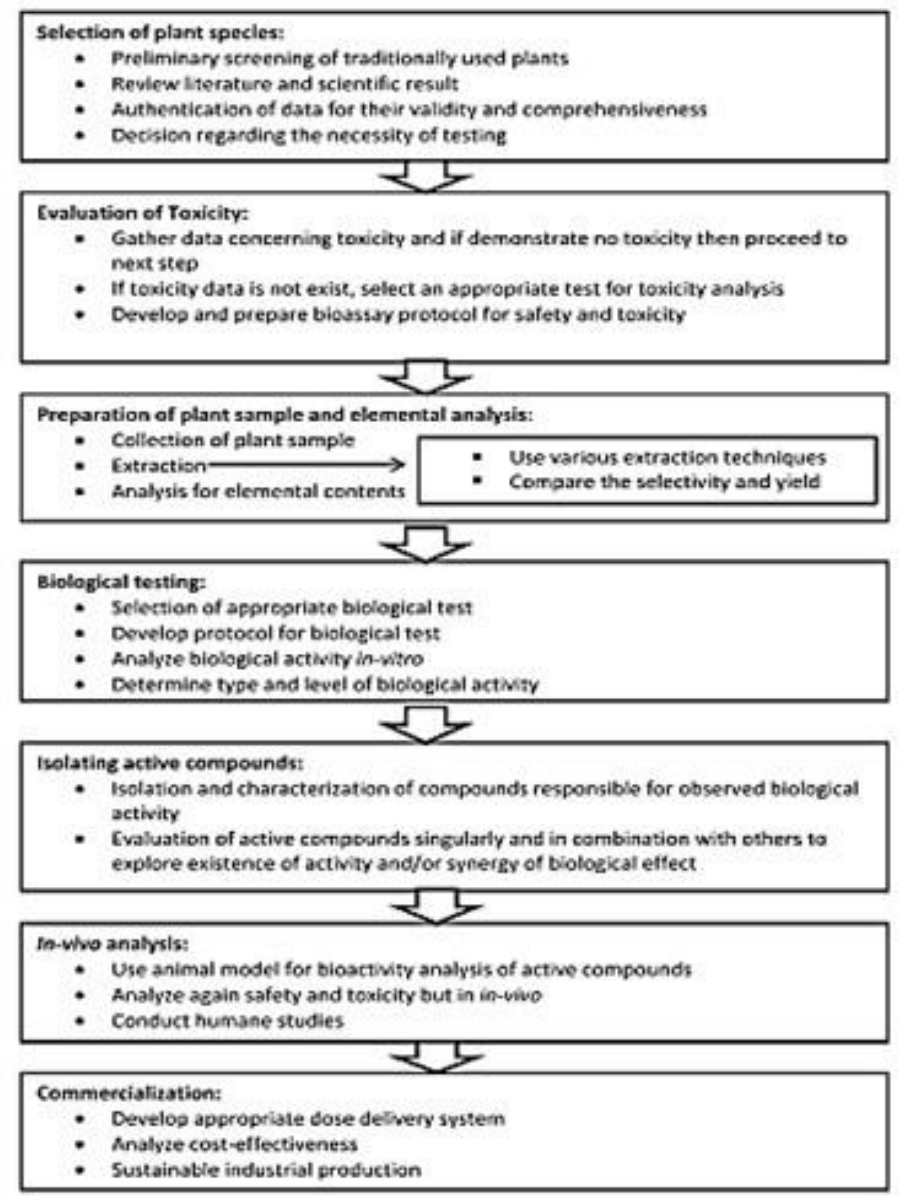


Figure 1 General approach in extraction, isolation and characterization of bioactive compounds.
Figure adapted from (1).

Water	Ethanol	Methanol	Chloroform	Ether	Acetone
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonol	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxyllines			
Polypeptides	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			
		Lactones			
		Flavones			
		Phenones			
		Polyphenols			

CONVENTIONAL METHODS FOR PLANT EXTRACTION

SOXHLET EXTRACTION

- Soxhlet extraction was first used for lipids, however, it not limited any more.
- A dry sample is placed in a thimble, which is further placed in a **distillation** flask containing the solvent of interest.
- Siphon unloads the solution back in the distillation flask.

Contd...

- The process is repeated until the final compound of interest is extracted

MACERATION

- Maceration is commonly used in homemade **tonics**.
- It is popular and used to obtain essential oil and bioactive compounds.
- The plant material is ground into smaller particles and mixed with a solvent.

Contd…

- Then the **menstruum** (solvent) is added to a closed vessel and the liquid is strained to recover the solid residues.

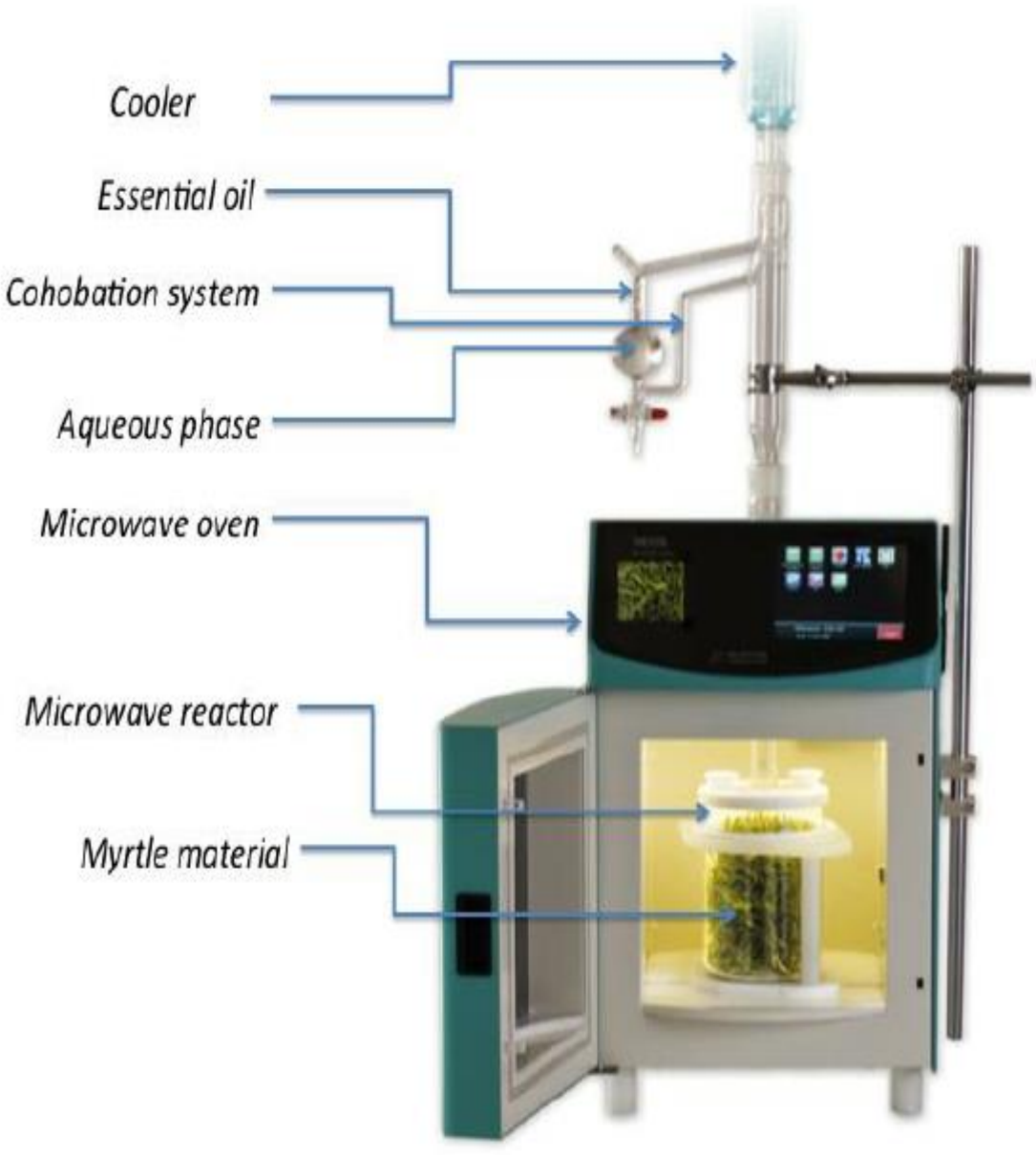
- The strained liquid is separated from impurities by filtration.

HYDRO DISTILLATION

- Hydro distillation is commonly used for essential oils from plants, which can be done via water, steam or direct steam distillation.

Contd...

- In this method, the **plant material** is boiled in water and direct steam is injected.
- Indirect cooling condenses the vapour and the separator separates the oil and bioactive compounds from the water.
- This method is not used for thermolabile compounds.



NON-CONVENTIONAL METHODS FOR PLANT EXTRACTION

- There are quite a few modern nonconventional methods which are preferred compared to the conventional methods.

- Some of the key modern methods are supercritical fluid extraction (SFE), **pressurized** liquid extraction (PLE), Microwave assisted extraction (MAE), Enzyme assisted extraction (EAE), pulsed electric field extraction (PEF) and Ultra sound assisted extraction (UAE).

Contd...

ULTRA SOUND ASSISTED EXTRACTION (UAE)

- Ultrasound passes through a medium by creating cavitation, i.e. compression and expansion.
- **Liquid** materials can be exploited using this cavitation effect.
- UAE facilitates the leaching of organic and inorganic compounds from the plant matrix, by intensifying the mass transfer.

Contd...

- **Ultrasound** extraction is found to be suitable for bioactive compounds from herbal plants.

- It is effective for achieving efficient mixing, faster energy transfer, reduced thermal gradients and selective extraction.

PULSED-ELECTRIC FIELD EXTRACTION (PEF)

- PEF treatment is reported to be useful for improving the pressing, extraction and diffusion process.
- PEF mechanism works on the principle of the destruction of cell membrane structure for enhanced extraction and decreased extraction time.
- PEF treatment is applied to improve the release of **intracellular** compounds by increasing the cell membrane permeability.

Contd...

- Based on the design of the treatment chamber, PEF process could either be operated in batch or continuous process.

- PEF treatment **parameters** such as field strength, specific energy, treatment time, temperature and property of the plant matrix are responsible for the effectiveness of the treatment.

- PEF treatments at 1kV/cm and 7kJ/kg have been used to extract betanin from beetroots.

- Moreover, recovery of phytosterols from maize was increased by 33% and isoflavonoids from soybeans were increased by 20%.

Contd...

- Application of PEF on grape skins before maceration has been reported to reduce the overall time required with improved stability.

ENZYME ASSISTED EXTRACTION (EAE)

- This method is widely accepted and used as it is novel, **non-toxic** and non-inflammable.

- In some plant matrices, phytochemicals are present in the cell cytoplasm and retained by hydrogen or hydrophobic bonding, which are not accessible by solvent extraction.

- The addition of specific enzymes such as cellulose, amylase, pectinase as a pretreatment enhances the recovery by breaking the cell wall and hydrolyzing the structural polysaccharides and lipids.

- Extraction of oils from various seeds can be achieved using **enzyme** assisted aqueous extraction.
- The extracted oils were found to contain a higher amount of free fatty acids and phosphorus content compared to the conventional methods.
- Enzyme composition, concentration, particle size and hydrolysis time are considered as key factors of EAE.

IDENTIFICATION AND CHARACTERIZATION

- Bioactive compounds could be isolated using chromatography techniques, such as TLC, column, flash, Sephadex and HPLC.
- The pure compound's structure is determined and then their biological activity.
- **Non-chromatographic** techniques include immunoassay, monoclonal antibodies, phytochemical and Fourier-transform infrared spectroscopy (FTIR).

Gas chromatography–mass spectrometry is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample.

High-Performance Thin-Layer Chromatography (HPTLC) instrument has attached to computer and data recording devices. The development of spots is viewed as peaks at wavelengths of selected UV regions. The height and the area of the peaks are determined by the instrument and recorded as a percentage.

Secondary metabolite	Name of test	Methodology	Result(s)
1) Alkaloid	Dragendorff's test	Spot a drop of extract on a small piece of precoated TLC plate. Spray the plate with Dragendorff's reagent.	Orange spot
	Wagner test	Add 2ml filtrate with 1% HCl + steam. Then add 1ml of the solution with 6 drops of Wagner's reagent.	Brownish-red precipitate
	TLC method 1	Solvent system: Chloroform: methanol: 25% ammonia (8:2:0.5). Spots can be detected after spraying with Dragendorff reagent	Orange spot
	TLC method 2	Wet the powdered test samples with a half diluted NH_4OH and lixiviated with EtOAc for 24hr at room temperature. Separate the organic phase from the acidified filtrate and basify with NH_4OH (pH 11-12). Then extract it with chloroform (3X), condense by evaporation and use for chromatography. Separate the alkaloid spots using the solvent mixture chloroform and methanol (15:1). Spray the spots with Dragendorff's reagent.	Orange spot
2) Anthraquinone	Borntrager's test	Heat about 50mg of extract with 1ml 10% ferric chloride solution and 1ml of concentrated hydrochloric acid. Cool the extract and filter. Shake the filtrate with equal amount of diethyl ether. Further extract the ether extract with strong ammonia.	Pink or deep red coloration of aqueous layer
	Borntrager's test	Add 1 ml of dilute (10 %) ammonia to 2 ml of chloroform extract.	A pink-red color in the ammoniacal (lower) layer
3)Cardiac glycosides	Kellar – Kiliani test	Add 2ml filtrate with 1ml of glacial acetic acid, 1ml ferric chloride and 1ml concentrated sulphuric acid.	Green-blue coloration of solution
	Kellar- Kiliani test	Dissolve 50 mg of methanolic extract in 2 ml of chloroform. Add H_2SO4 to form a layer.	Brown ring at interphase
	TLC method	Extract the powdered test samples with 70% EtOH on rotary shaker (180 thaws/min) for 10hr. Add 70% lead acetate to the filtrate and centrifuge at 5000rpm/10 min. Further centrifuge the supernatant by adding 6.3% Na_2CO_3 at 10000 rpm/10min. Dry the retained supernatant and redissolved in chloroform and use for chromatography. Separate the glycosides using EtOAc-MeOH-H_2O (80:10:10) solvent mixture.	The color and hR_f values of these spots can be recorded under ultraviolet (UV254 nm) light
4) Flavonoid	Shinoda test	To 2-3ml of methanolic extract, add a piece of magnesium ribbon and 1ml of concentrated hydrochloric acid.	Pink red or red coloration of the solution
	TLC method	Extract 1g powdered test samples with 10ml methanol on water bath (60°C/ 5min). Condense the filtrate by evaporation, and add a mixture of water and EtOAc (10:1 mL), and mix thoroughly. Retain the EtOAc phase and use for	The color and hRf values of these spots can be recorded under ultraviolet

Table 2 Summary of phytochemical screening of bioactive compounds

		chromatography. Separate the flavonoid spots using chloroform and methanol (19:1) solvent mixture.	(UV254nm) light
	NaOH test	Treat the extract with dilute NaOH, followed by addition of dilute HCl.	A yellow solution with NaOH, turns colorless with dilute HCl
5) Phenol	Phenol test	Spot the extract on a filter paper. Add a drop of phoshomolybdic acid reagent and expose to ammonia vapors.	Blue coloration of the spot
6) Phlobatannin	-	2 ml extract was boiled with 2 ml of 1% hydrochloric acid HCl.	Formation of red precipitates
7) Pyrrolizidine alkaloid	-	Prepare 1ml of oxidizing agent, consisting of 0.01ml hydrogen peroxide (30% w/v) stabilized with tetrasodium pyrophosphate (20mg/ml) and made up to 20ml with isoamylacetate, and add to 1ml of plant extract. Vortex the sample and add 0.25ml acetic anhydride before heating the sample at 60°C for 50-70s. Cool the samples to room temperature. Add 1ml of Ehrlich reagent and place the test tubes in water bath (60°C) for 5min. Measure the absorbance at 562nm. The method of Holstege et al. (1995) should be used to confirm results of the screening method	Peaks were compared with the GC–MS library
8) Reducing sugar	Fehling test	Add 25ml of diluted sulphuric acid (H_2SO_4) to 5ml of water extract in a test tube and boil for 15mins. Then cool it and neutralize with 10% sodium hydroxide to pH 7 and 5ml of Fehling solution.	Brick red precipitate
9) Saponin	Frothing test / Foam test	Add 0.5ml of filtrate with 5ml of distilled water and shake well.	Persistence of frothing
	TLC method	Extract two grams of powdered test samples with 10 ml 70% EtOH by refluxing for 10 min. Condense the filtrate, enrich with saturated *n*-BuOH, and mix thoroughly. Retain the butanol, condense and use for chromatography. Separate the saponins using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. Expose the chromatogram to the iodine vapors.	The colour (yellow) and hRf values of these spots were recorded by exposing chromatogram to the iodine vapours
10) Steroid	Liebermann-Burchardt test	To 1ml of methanolic extract, add 1ml of chloroform, 2-3ml of acetic anhydride, 1 to 2 drops of concentrated sulphuric acid.	Dark green coloration
	-	To 1 ml of extract, add 2 ml acetic anhydride and 2 ml concentrated sulphuric acid H2SO4.	Color change to blue or green
	TLC method	Extract two grams of powdered test samples with 10ml methanol in water bath (80°C/15 min). Use the condensed filtrate for chromatography. The sterols can be separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The color and hRf values of these spots can be recorded under visible light after spraying the plates with anisaldehyde-sulphuric acid reagent and heating (100°C/6 min)	The color (Greenish black to Pinkish black) and hR_f values of these spots can be recorded under visible light

CONCLUSION

- The increasing demand for plant based bioactive compounds has developed continuous extraction methods.
- The development of non-conventional extraction procedures and **chromatography** advancements has resulted in efficient extraction and isolation of bioactive compounds.

Thank You For Your Attention