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Isolation of Natural Products

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Abstract
Plant had been used as medicine in ancient. Now day's Pharmaceutical companies start processing of medicinal and aromatic plants in their formulation by using extraction of active components. Extraction of plant components likes volatile, Essential or ethereal oils and mixtures composed of volatile liquid and solid compounds depend upon their composition and their boiling point. Now days there are several processes like distillation, enfleurage, maceration, expression, solvent extraction and fluid extraction are available for extraction of plant component. This review also summarizes the characters of phytoconstituents, choice of solvents, influence of solvents, extraction strategy, procedures for extraction of herbal drugs and treatment of drug residue after extraction.

Key Words
Secondary metabolites, Phytoconstituents, Enflurage.

Introduction
Natural products are secondary metabolites which are derived from herb or animal sources. Natural products are chemical compound found in nature and they have pharmacological and biological activity. Natural products are generally used in drug discovery and drug design. Separation of a single molecular entity is very difficult from complex mixtures contain fats, oils, alkaloid, tannins and glycoside. In 1803 the first alkaloid, nicotine and then morphine, strychnine, emetine and many others were separated. This review compiles the recent literature with special focus on various approaches for extraction. This review also summarizes choice of solvent, interfering compound and strategies involve in extraction.

Isolation of Natural Products

As time passes new separation techniques and analysis are being introduced to separate different nature of compounds like alkaloids, glycosides, steroids, saponins, tannins, flavonoids etc. Carbohydrates, fats, and proteins are considered more valuable as they are having dietetic importance.[1, 2] Many starches and gums are used in pharmacy but lack any marked pharmacological action; are used as binder, viscosity builder and as hydrocolloid to increase the stability of emulsions and suspensions.[1]

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After GATT there is a great surge to find out immense potential of traditional and other herbal drugs to prevent and cure diseases. Several extraction, fractionation/separation and isolation methods are developed after which isolation of the active moiety and their chemical examination is performed. Microwave extraction, sonication, lyophilization, spray drying and vacuum drying have also been employed with good results. Generally the extraction is based on pharmacological activity rather than chemical nature of compounds. Further studies are carried out after the isolation of the active moiety to confirm the compound.[3] The phytochemical investigation of a plant involves the selection, collection, identification and authentication, extraction of the plant material (first fractionation), fractionation/separation (second fractionation) and isolation (third fractionation) of the constituents, characterization of the isolated compounds and investigation of the biosynthetic pathways of particular compound, quantitative evaluations and pharmacological activities.[4]

Terms and Definitions

The extraction of drug represents either solid-solid or liquid-liquid extraction. Some common terms used in phytochemistry are given in Table 1.

Principle
Extraction processes for drugs can depends on the partition of component between solvent phase and solid residual and dependent on diffusion of

component. Solvent volume is used such as the final concentration gradient between miscella and residue has become zero which is an equilibrium stage.[6] The position of the equilibrium depends on properties of the drug nature and type of drug, quantity, degree of comminution, solvent selectivity, solvent quantity and moisture content. Factors which affect the extraction are quantity and nature of drug, degree of size reduction, moisture content, volume and nature of solvents, mixing ratios of solvents, method for preparation of solution from intact cells, method for preparation of solution from lysed cells, imbibition by solvent, rate of equilibrium establishment, temperature, pH of the extracting solvent, interaction between dissolved components, polarity of the solvent mixture (s), process governing separation, mixture ratio of solvent and herb, dissolution from lysed cells, penetration of solvent and swelling of drug plant material, movement of constituents out from intact cells and interaction of dissolved constituents with insoluble support material of plants.[2,5,7]

Strategy for Investigation

Strategy for investigation is divided into following stages:

1. Pre-extraction investigations
    a. Collection, identification, selection and authentication of the plant material
    b. Drying and grinding
    c. Nature of constituents or secondary metabolites
    d. Solvents for extraction
    e. Interfering compounds
2. Extraction method
    a. General
    b. Miscellaneous methods
    c. Extraction of alkaloid, sesquiterpene lactone and cardiac glycoside, flavonoids, other polyphenols, sterols, saponins, carbohydrate
3. Fractionation, purification and isolation
4. Detection (Reagents for detection of phytochemical groups as alkaloid, sesquiterpene lactone and cardiac glycoside, flavonoids, other polyphenols, sterols, saponins, carbohydrate)
5. Solvent recycling

Selection, collection and identification and authentication of the plant material

The plant material should be properly authenticated to get desired component with high yield and reproducible results. The running of drug-discovery programs on randomly collected/selected plants is less economic.

Selection of plant

Generally plant selection involves a deep literature survey of the floristic diversity. Selection of plant material is performed by different approaches

1. Totally random selection.
2. Specific selection using ethnopharmacological reports.
3. By restricting the plants of interest to group based on chemotaxonomic, geographical, or compound structural - type preferences.
4. Computer-based selection method or Literature lnformation Selection Technique (L.I.S.T) using the NAPRALERT database (Novel approach involves correlation of biological activity, botanical facts, and chemotaxonomic information.[8]

Collection

During collection of plant, it should be kept in mind that the specimens to be studied should be healthy. The microbial growth or other microbial infections may change the metabolites produced by the specimen, e.g.by phytoalexin formation.[2, 3]

Factors Influencing the Concentration Levels and Kinds of Secondary Metabolites

Some of the factors that affect the concentration levels and kinds of secondary metabolites are site altitude, plant age, climate, soil type, time of collection, species etc. Chemical composition of herms also varies depending on species and environmental factors.[4]

Identification and authentication

After collection, the plant material should be identified or authenticated by a taxonomist. At least three specimens should be prepared. One of these samples should be deposited in a local national herbarium, and the others should be deposited in a specialist museum or herbarium and kept in an appropriate protected place for future reference.

Following details should be mentioned on herbarium

1. Place
2. Altitude
3. Environment
4. Characteristics
5. Chemical constituents
6. Part of plant taken
7. Season

Above information is of vital importance in those cases where a recollection of the plant material is necessary and it is beneficial for researchers to reproduce their work in future. An erroneous identification of a plant sample may lead to confusion in scientific literature.[9]

Drying and grinding

Depending on the nature of secondary metabolite, either material is directly processed like fresh leaves of Mentha spicata to obtain peppermint or material is processed after drying like Curcuma longa to obtain curcumin. Mostly plant material is dried in shade at room temperature or in hot air oven at not more than 30°C.1, 5 Sunlight consists of ultraviolet radiation which may cause chemical degradation/reactions and may result to giving rise compound artifacts, so direct contact of sunlight is avoided. Fungal growth at elevated temperatures by fermentation and aeration may change the content and nature of secondary metabolites. Fresh plant material should be immediately extracted with solvent to prevent enzymatic processes or reactions that start after the plant is collected or during grinding. Hydrolysis of constituents such as iridoid and flavonoid glycosides may lead to change in pH and result in decomposition or rearrangements of compound. These reactions may be prevented by soaking the sample in alcohol (methanol or ethanol) which denature the plant enzymes which are responsible for degradation.7 Use of buffers also prevents degradation. When studying essential oils, volatilization of metabolites should be avoided to prevent loss. On prolong storage the constituents of plant material may undergo decomposition like in herb Antireha putminosa which loses 50% its alkaloid after 2 months of storage, whereas some flavonoid glycoside may get hydrolyzed and degradation or detection of any changes can be confirmed by TLC.[10]

Comminution

Herb must be washed properly to remove extraneous matter like san or dust, spider silk etc. Dust may clog the percolators. Generally sand is removed by pneumatic method which involves magnetic removal of metals followed by a preliminary sieving.[3, 6] Shredding is the first stage which is then followed by comminution. Comminution is performed to obtain high yield. Marked differences in the particle size may prolong the extraction times. Small particle size of material will yield high extraction values in less duration.[7]

Characters of Phytoconstituents

The basic knowledge of nature and characteristics of phytoconstituents is essential to select method and solvent for extraction. Nature of phytoconstituents involves polarity, pH, thermostability etc.

Polarity

As a doctorine “like dissolves like”. Polar components are soluble in polar solvent and non-polar components are soluble in nonpolar solvents. Solvent selection depends on either nature of phytoconstituents directly or extraction of component followed by removal of interference first (example for curcumin extraction defatting is done first and then extraction is done by methanol and chloroform). It is rudimentary to study about the relationship between the extraction method applied and the physicochemical properties of the substances to be extracted.[11]

pH

The pH of solvents affects the extent of extraction of components. Non- polar alkaloids can’t be extracted into aqueous system but can be extracted into polar aqueous acid due to their basic nature and salt formation in acid. Fatty acids, phenols and other acidic phytochemicals are extracted using solvents at alkaline pH.6 The compounds should not break down at employed pH values, e.g. esters are prone to hydrolysis in alkali and glycosides lose the sugar moiety in acid.[7]

Thermostability

Normally solubility increases with raise in temperature. Higher temperatures facilitate penetration of the solvent into the cellular structures of herbs. Thermolabile components are sensitive to to higher temperatures. The artifacts may arise in presence of solvent/components on heating which may degrade the biologically active moiety or may exert toxicity or may create separation problem.[9]

Choice of Solvents

Usually secondary metabolites have different degrees of polarity so the solvent(s) should be chosen for the extraction should be considered carefully to ensure dissolution of secondary metabolites under study.

Solvent should have following properties:

1. Easy to remove
2. Inert

3. Nontoxic
4. Not easily inflammable
5. No interaction or less chemical interaction

Solvent (mixture of solvent) is employed to dissolve the secondary metabolite and finally to diffuse out the dissolved solute into bulk solvent phase.

Solvent employed are:

1. Polar: Water
2. Non-polar: Petroleum ether, chloroform, Diethyl ether
3. Semipolar: Ethanol, Acetone
4. Azetropic mixtures

Polar Solvents

The polar components like polysaccharides, phenols, aldehydes, ketones, amines, and other oxygen containing compounds dissolve in water due to formation of hydrogen bonding. The solubility of aliphatic alcohol increases the solubility of the compound in water decreases. Additional polar groups are present in the molecule, as found in propylene glycol, glycerin, and tartaric acid, water solubility increases greatly due to addition of polar groups. Branching of the carbon chain reduces the nonpolar effect and leads to increased water solubility (tertiary butyl alcohol is miscible in all proportions with water, whereas n-butyl alcohol dissolves to the extent of about 8 g/100 ml of water at 20°C). The polar solvents such as water act as solvents according to the following mechanisms:

1. Normally polar solvents have high dielectric constant which reduces the force of attraction between oppositely charged ions in crystals such as sodium chloride or molecule. Polar solvent like water has a dielectric constant of 80 while which dissolve polar component rapidly than non-polar solvent chloroform, which has a dielectric constant of 5 and due to low dielectric constant, ionic compounds are practically insoluble in non-polar organic solvents.
2. Polar solvents break covalent bonds of potentially strong electrolytes by acid-base reactions since these solvents are amphiprotic. For example, water brings about the ionization of HCI as follows:

a. Weak organic acids are not ionized appreciably by water

b. Their partial solubility is attributed instead to the hydrogen bond formation by with water. Phenols and carboxylic acids, however, are readily dissolved in solutions of strong bases.

3. Polar solvents has property of dipole interaction forces, particularly hydrogen-bond formation dut to which solvating molecules and ions become soluble and which leads to the solubility of the compound. The solubility of sodium salt of oleic acid and water is due to ion-dipole interaction.[11]

Non-polar Solvents

Non-polar solvents have low dielectric constants and dissolve non-polar solutes with similar internal pressures through induced dipole interactions. Ionic and polar solutes are insoluble or slightly soluble in non-polar solvents. Weak Van-Der-Waals and London type of forces are responsible for the solubility of molecules.

Semi-polar Solvents

Semi-polar solvents like ketones and alcohols can induce a certain degree of polarity in non-polar solvent like benzene is readily polarizable, becomes soluble in alcohol. Semi-polar compounds act as intermediate solvents which bring about miscibility of polar and non-polar liquids.

Azeotropic Mixtures

Azeotropes are mixture of different solvent with varying polarity which has near boiling points. These are usually either binary or ternary mixtures with the ratio of their mixture (wt %) of component 1 soluble with wt % of component 2 and ternary azeotropic mixtures with the respective concentration of their mixture (mol %) of component 1 miscible with mol % of component 2 and component 3.8 They affect the dissolution properties and degree of extraction of extractable matters. To utilize this phenomenon fully it is recommended that the composition of the menstrum be chosen so that a binary or ternary azeotropic mixture is produced.10 This has the advantage that upon concentration of the extracts, the solvent boils constantly and the condensate, perhaps after a small correction by replacement of components preferentially retained in the drug residue, can be reused. Azeotropic mixtures have great potential to extract active phytochemical metabolites from the crude drugs depending on their varied chemical nature as they can extract large numbers of constituents based on its nature.

Influence of Solvents

A component may behave like strong electrolyte or non-electrolyte, depending on pH of solution. Precipitation of components occurs, when the pH of solution is adjusted to such a value at which un-

ionized molecules are produced in sufficient concentration to exceed its solubility.[4]

Solvent-Solute interactions

Polar solvents like water is a good solvent for salts, sugars etc while non-polar solvents like mineral oil and benzene are often solvents for substances that are normally only slightly soluble in water. It proves the doctorine of "like dissolves like".[9]

Combined Effect of pH and Solvents

The solvent affects the solubility of a weak electrolyte in a buffered solution in two ways,

I. The addition of alcohol to a buffered aqueous solution of a weak electrolyte increases the solubility of the un-ionized species by adjusting the polarity of the solvent to a more favourable value.

II. Being less polar than water, alcohol decreases the dissociation of a weak electrolyte, and the solubility of the drug goes down as the dissociation constant is decreased (pKa is increased).[13]

Solvents, Problems and Limitations

The secondary metabolites must dissolve in solvent chosen for the extraction. The solvent chosen for the extraction must have following qualities

1. It must dissolve the secondary metabolites
2. It should be easy to remove.
3. It should be inert.
4. It should be nontoxic.
5. It should not be easily flammable.
6. It should not form any type of unstable substance during extraction or mixing.
7. Solvents should be distilled or even double distilled prior to use if they are of low or unknown quality.
8. Solvent must be free from plasticizers like dialkyl phthalate, tri-n-butyl acetyl citrate and tri-butyl phosphate which are commonly found as impurities in solvents and mat impart stability problem.[6,9,12]

Extraction of Plant Material

There is no single perfect method for extraction, purification and isolation of compound. The first fractionation step is general extraction of secondary metabolite which is done either by using single „all-purpose" solvents such as methanol or by using petroleum ether to defatted the material. Methanol dissolves most of the secondary metabolites and enhancing their release from cellular matrix/cell sufrace.[9,10] Filtration or centrifugation is the first basic technique used to separate insoluble material and filtrate that contain dissolved secondary metabolite. The choice of extraction procedure and extraction solvent depends on the physicochemical nature of secondary metabolite, nature of plant material (fresh parts, dried parts) and their physical state (particle size)**.** Fresh plants (leaves) are first homogenized or macerated with alcohol prior to extraction.[13] An idea about properties of interfering compound provides best criteria for solvent selection for extraction and to select method of extraction. So prior to extraction a major emphasis must be given on nature of interfering compounds.

Interfering Compounds

Many naturally occurring compounds may interfere with the isolation and purification of a desired bioactive plant constituent like lipids, pigments, tannins, plasticizers, silicon etc.

Effect of Solvent, Solvent Mixtures and Solution on Extraction

Solvent or the extraction agents used in the preparation of phyto pharmaceuticals must be suitable for dissolving the important therapeutic drug constituents and thus for separating them from the substances containing the drugs which are to be extracted.10 ln pharmaceutical technology, the extraction agent or solvent is known as menstrum and the extract solution separated from the residual insoluble drug plant material is called miscella. Menstrum are therefore solvents which readily dissolve and often also have certain selectivity for the extracted substances. For example, bitter principles, mucin, pigments, resins, etc. should (if at all possible) not be dissolved in the extraction of vegetable oils.[8, 9, 12] According to the customary definition in this technology solvents are, under normal conditions, volatile, usually organic liquids capable of dissolving other gaseous, liquid or solid substances without either themselves or the dissolved substance being chemically altered. Ln pharmaceutical technology, this is not always the case, as solvents such as oils that are not volatile under normal conditions are also used.[13] The only inorganic solvents used in pharmacy are water and CO2, although liquids such as ammonia, sulphur dioxide and hydrocyanic acid solutions are used for special purposes in pharmaceutical processing.[14] Water, pure organic liquids and mixtures of organic liquids with water or with other organic\ liquids are used as extraction solvents. These organic liquids are nearly always hydrocarbons and their derivatives

such as halogenated hydrocarbons, alcohol, esters, ketones, ethers, oils, etc. Selectivity, ease of handling, econorny, protection of the environment and safety are major factors to be considered in the choice of a suitable solvent or of a mixture of several solvents.[6, 13, 14.] The last two factors in particular are becoming increasingly important due to the legislators sharpened attention to them. This has become so critical that many manufacturers are making serious attempts to design extraction processes so that the smallest possible number of 'safe' liquids, e.g. water, lower alcohol and in special areas, super critical gases are used. It must not be forgotten here that traditional plant extracts must satisfy the requirements of the pharmacopoeias. This is usually possible only when the specified menstrum is used. A degree of choice is offered by using cheaper methanol instead of expensive ethanol (on which duties have to be paid) if its polarity is equal to that of the required ethanol-water mixture. As a rule of thumb, a 10% higher methanol concentration roughly corresponds to the required ethanol concentration.[16] One essential consideration in the choice of suitable solvents is the question of whether the menstrum remains wholly or partly in the end product. lf this is the case, physiologically harmless solvents must be used. lf the end-product is free of solvent, as is the case with dried extracts, or if it still contains only harmless components from the originally used solvent mixture after further processing of the miscella, then for reasons of selectivity and economy the choice can be made without considering the physiological properties of the mixture used. [16,17]

Extraction Strategy

Isolation of compound

The 3 stages are basic

1. First step of extraction involves solvent penetration into herb cells/tissues, solubilization of secondary metabolites and finally release the dissolved secondary metabolites in solvent of extraction. Solvents of varying polarity are used alone or in combinations for extraction depend on component. So a large proportion of the unwanted material is removed. (Maceration, Digestion, Decoction, Soxhlet extraction, Supercritical chromatography, Hydro-distillation, Enflurage, Eculle, Supercritical fluid chromatography)
2. Second step is fractionation with subsequent analysis: Either open silica column or counter current distribution/liquid-liquid extraction is used to separate or fractionation of components. (Distillation, Sublimation, Evaporation, Fractional crystallization, fractional distillation, Sublimation, Fractional crystallization, fractional distillation, GC, CCD)
3. The third final stage is achieved by HPLC or TLC which involves separation of desired component in adequate purity (TLC, GC, GLC, Mass, NMR, UV, Fluorimerty)

Extraction is followed either by

1. Powdered dried material is directly extracted to achieve extract
2. Or first defeat the material and then extraction of desired component
3. Or fresh plants (e.g. leaves) can be homogenized or macerated with alcohol

Selection of extraction method depends on

1. Nature of component
2. Nature of material to be used
3. Solvent system available

Techniques used to enhance extraction

1. Ultrasound may enhance the extraction process for some plant materials, eg., the preparation of a 50% ethanolic solution of opium for the assay of alkaloid.
2. Use of microwaves can also enhance extraction.
3. By altering pH
4. By stirring
5. By reducing the particle size
6. By changing the polarity of solvents

Procedures for Extraction of Herbal Drugs

During the extraction of the herbal drugs the rinsing of extractive substances out of disintegrated plant cells, swelling of the drug plant material in order to increase the permeability of the cell walls, Penetration of the solvent into the plant cells and swelling of the cells, Dissolution of the extractive substances, Diffusion of the dissolved extractive substances out of the plant cell and finally the dissolution of extractive substances out of intact plant cells by diffusion take place. It has been found that the liquorice root when extracted with the solvent (0.25% ammonia solution) it penetrates into the roots more rapidly and this process is accelerated by raising the temperature. The steeping and swelling process is strongly influenced by particle size and is more evident radically. Upon penetration into the plant material, the solvent becomes enriched with extractive substances and hence the highest content of extractive substances is found on the

solvent front. Some general points should be considered in terms of the extraction process, such as the overall characteristics of the secondary metabolites to be extracted (e.g. some glycosides are thermolabile or pH-sensitive). Although the normal practice is 'to apply 2iitanOarO technique to obtain a crude extract from a plant material, e.g. an acid-base shakeout to prepare an alkaloidal extract, because of the structural diversity within a given natural product group and their possible special requirements, it is advisable to consult specific reviews, .papers, and books in order to prevent the avoidable loss of desired bioactive metabolites caused by the use of an inappropriate extraction technique. The most simple extraction processes employed may be classified as follows: extraction with organic solvents: percolation, maceration, and extraction using a Soxhlet apparatus; and extraction with water: infusion, decoction, and steam distillation.[1,9,15] The most popular method of extraction is to use a liquid solvent at atmospheric pressure, possibly with the application of heat. Other methods include steam distillation, supercritical fluid extraction and the use of liquefied gases under moderate pressure. The choice of method depends on the factors listed above as well as the intrinsic advantages and disadvantages of the procedures.[18],

Supercritical fluid extraction

The process of separation of one component which is extracting from matrix by using supercritical fluid is known as supercritical fluid separation. Supercritical fluids showed property intermediate between those of the liquid and gaseous phases, for any substance it is a condition above the critical temperature and pressure.SFE offers many advantages as follows:

1. It leads to lower solvent usage
2. Controllable selectivity
3. Cleaner extracts and less thermal degradation as compared to conventional solvent extraction and steam distillation methods,

Super critical carbon dioxide (SCO2) - with its particularly attractive properties such as non-toxicity, non-flammability, non-corrosiveness, chemical inertness, low critical temperature (304"K), moderately low critical pressure (73 atm), easy availability, co-effectiveness and environmental acceptability is the preferred solvent for many super critical extractions. Liquid carbon dioxide is completely miscible with components of essential oils like aldehyde, ketones, esters and alcohols. At same time, proteins, starches, mineral salts and water are insoluble in liquid carbon dioxide. Essential oils obtained by liquid carbon dioxide extraction are superior to that obtained through steam distillation and solvent extraction. Extraction of several natural products such as pyrethrins from chrysanthemum flower, essential oils from anise, caraway, clove, star anise, cinnamon and ginger are increasingly done by this process.[3,9,16]

Solid phase extraction

Solid phase extraction is process of separation of dissolve and suspended component from liquid mixture by using another component in the mixture according to their physical and chemical property.9

Distillation

Distillation may be defined as separation of components of a mixture of two or more liquids by virtue of difference in their vapor pressure. There are three systems of distillation-

- Hydro distillation
- Hydro-steam distillation
- Steam distillation

Hydro distillation-Hydro distillation is the oldest method being used for separation of essential oil. In this method plant material is contact with boiling water in a crude metallic distillation unit. This process use principle of osmotic press principle of osmotic pressure to diffuse oil from the oil glands. The essential oil of a plant consists of many compounds which generally boil between 150° to 300° C. The vapors pass through a coiled tube contained in a water bath and condensate is obtained at the bottom of the condenser tube. The disadvantages are that the heat is difficult to control and hence the rate of distillation is variable. Also the possibility exists for local overheating and "burning" of the charge which can lead to poorer quality oil.

Hydro-steam distillation

To overcome the drawback of water distillation, modifications in techniques was developed. In this technique plant material is supported on a perforated grid or screen inserted at some distance above bottom of still. Water filled below the grid is heated which produce saturated and wet steam; produced steam pass through plant material and vaporized essential oil.

Steam distillation

A process of extracting essential oils from plant products through a heating and evaporation process is known as steam distillation. Steam distillation is a popular method for the extraction of volatile oils (essential oils) from plant material. This can be carried out in a number of ways. One method is to mix the plant material with water and to heat to boiling (distillation with water). The vapors are collected and allowed to condense, and the oil separated from the water. . It resembles hydro-steam distillation except that no water is kept in bottom of still. This method is efficient and gives higher yields. However, it is not generally employed to delicate flowers. To maximize the yields of the oils, precautions must be taken to ensure efficient condensation of the steam and vaporized oil and collection of the condensate in such a way as to prevent loss of the volatile material. However, to avoid risk of explosion, a completely closed system must not be used. The advantages of this type of "dry" steam distillation are that it is relatively rapid, therefore charging and emptying the still is much faster and energy consumption is lower. The rapid distillation is also less likely to damage those oils which contain reactive compounds, e.g. esters. However, it cannot be used where the oil contains hydrolysable components such as esters or those that are easily.[8,14]

Maceration (Extraction with hot fat)

Maceration is process of extraction with hot oil or fat. In maceration, oil cells of fragrant flowers are ruptured by immersion in a hot fat or oil at 60-70°C which in turn absorbs essential oils. Fat is separated from spent flowers and reused for absorbing fragrance from next batch of fresh flowers. Fat retained by flowers is recovered by hydraulic pressing. Resultant perfumed pomade is frequently marketed as such but is often extracted with strong alcohol to yield extracts. This is very much the same technique used in solvent extractions, where solvents are used instead of the hot oil as used in maceration.[16,18]

Enfleurage (Extraction with cold fat)

Enfleurage is the process of extraction of fragrance by absorbing it from flowers in contact with cold fats. This process is adopted for fragrant flowers of jasmine and tuberose, which continue to manifest their characteristic fragrance even in plucked condition. Fats should be saturated and odorless to prevent entrance of fat odors. Refined lard or beef suet are preferred. Fat is thinly layered on both sides of a glass plate supported on a rectangular wooden frame or chassis. Fresh fragrant flowers are lightly layered on fat coated chassis.

Enfleurage gives a much greater yield of flower oil than other methods. Despite this advantage, enfleurage has lately been replaced by extraction with volatile solvents because enfleurage is a very delicate and lengthy process requiring much experience and labour.[4,8]

Extraction with volatile solvents

Principle of extraction with volatile solvents is simple. Fresh flowers are charged into specially constructed extractors and extracted systematically at room temperature, with a carefully purified solvent usually petroleum ether. Solvent penetrates flowers and dissolve natural flower perfume together with some waxes and albumins and colouring matter. Solution is subsequently pumped into an evaporator and concentrated at a low temperature. After the solvent is completely driven off in vacuum, concentrated flower oil is obtained. Thus, temperature applied during entire process is kept at a minimum; live steam as in case of distillation, does not exert its action upon delicate constituents of flower oil. Compared with distilled oils, extracted flower oils more truly represent natural perfume as originally present in flowers.[10]

Expression

It is physical process in which pressure is applied to squeeze the oil out of the material or juice from plant. This was usually achieved by a tincture press. This method is employed when essential oils are thermo sensitive. It is used for isolating essential oils from lemon and orange peels. In general, expression involves squeezing any plant material at great pressures to press out oils or other liquids. The process is carried out by hand-operated presses or crushes in isolated rural areas or by gigantic mechanical presses in industrial centres.[9,15]

Infusion

Infusions are prepared by soaking a drug in water for a specialized period of time. The process can be either hot or cold, depending upon the type of the ingredients present as decomposition may occur at higher temperatures. Infusions are generally prepared for immediate use, as preservatives are absent. In some cases preservatives like alcohol are used and the infusions concentrated by boiling. The

term infusion are used for the preparations prepared from soft tissues like petals, leaves etc.

Percolation (Exhaustive Extraction)

Percolation in usually one of the most widespread methods for plant extraction since it does not require much manipulation or time. It is a continuous process in which the saturated solvent is constantly being displaced by fresh menstrum. Normally, percolation is not used as a continuous method because sample is steeled in solvent in the percolator for 24hrs (for up to three times), and then the extracted materials are collected and pooled.[9] In general process of percolation, particularly in the manufacture of concentrated preparations like liquid extracts, the following problems may arise:

a) If the active substances are thermo-labile, evaporation of large volume of dilute percolate, may result in partial loss of the active constituents

b) In the case of alcohol- water mixture, evaporation results in preferential vaporization of alcohol leaving behind an almost aqueous concentrate which may not be able to retain the extracted matter in solution and hence get precipitated. In such cases the modification in general process of percolation is required as given below,

Reserved Percolation

In this case the extraction is done through the general percolation procedure. At the last, the evaporation is done under reduced pressure in equipment like a Climbing evaporator to the consistency of a soft extract (semi solid) such that all the water is removed. This is then dissolved in the reserved portion which is strongly alcoholic and easily dissolves the evaporated portion with any risk of precipitation.[11]

Decoction

Decoctions are prepared in a similar manner to that of infusions but the ingredients are boiled with that of water for a specified period of time or till a definite volume is attained. The term decoction is used when the preparation is prepared using hard plant parts like root, bark, wood etc. Decoctions are usually the method of choice when working with tougher and more fibrous plants, barks and roots (and which have water soluble chemicals). Depending on the type of plant material used, strong decoctions are prepared in two general ways. The first involves boiling the mixture longer. This is usually indicated when working with larger woody pieces of bark. Longer boiling time, up to 2 hours or more, is sometimes necessary to break down, soften, and extract the larger pieces. Alternatively, when smaller woody pieces are used yet a stronger remedy is wanted, the decoction is prepared as above (boiling 20 minutes), then it is allowed to sit/soak overnight before straining out the herb. When straining, again, make sure to press on the cut herb pieces in the strainer to get as much moisture/decoction out of the herb pieces.[1, 7, 11, 17]

Ultrasound Extraction

Extraction of intracellular compounds by cell-lysis is done by using ultrasound. When sound wave propagate in liquid media results in high-pressure and low-pressure cycle. The main effects of ultrasound extraction can be summarized as:

- To increase the permeability of the cell walls
- To produce cavitations i.e. the spontaneous formation of bubbles in a liquid below its boiling point resulting from strong dynamic stressing
- To increase mechanical stressing of the cells so called interface friction

Treatment with the ultrasound plays a major role e.g. decomposition of the alkaloids in jaborandi leaves is observed after 30 s ultrasound treatment on the laboratory scale at 20KHz but in the case of foxglove leaves the content of digitalis glycosides fell when an ultrasound output representing the optimum formation of hydrogen peroxide during the extraction .10,18

Hot continuous extraction (Soxhlet extraction)

Soxhlet extraction method described by soxhlet in 1837.In this methods fat and oil from solid material is extracted by repeated washing with organic solvent under reflux. Organic solvent commonly used are hexane and petroleum ether. Disadvantage of this process are-polar lipid, long time involved large volumes of solvents, hazards of boiling solvents.[18]

Digestion

The in-gel digestion is part of the sample preparation for the mass spectrometric identification of proteins in course of proteomic analysis. The in-gel digestion primarily comprises the four steps destaining, reduction and alkylation (R&A) of the cysteines in the protein, proteolytic cleavage of the protein and extraction of the generated peptides.[9]

Destaining

Proteins which were separated by 1D or 2D PAGE are usually visualized by staining with dyes like Coomassie Brilliant Blue (CBB) or silver.[12]

Reduction and alkylation

The staining and destaining is often followed by the reduction and alkylation (r&a) of the cystines or cysteines potentially embodied in the protein. Hereby the disulfide bonds of the proteins are irreversibly broken up and the optimal unfolding of the tertiary structure is obtained. The reduction to the thiol is accomplished by the reaction with chemicals containing sulfhydryl or phosphine groups such as dithiothreitol (DTT) or tris-2-carboxyethylphosphine hydrochloride (TCEP).[2,5,10]

Digestion

The protein is cut enzymatically into a limited number of shorter fragments. These fragments are called peptides and allow for the identification of the protein with their characteristic mass and pattern. The serine protease trypsin is the most common enzyme used in protein analytics.[12,13,18]

Extraction

After finishing the digestion the peptides generated in this process have to be extracted from the gel matrix. This is accomplished by one or several extraction steps. Drawbacks for the in-gel digestion are the extended time need and the multiple processing steps making the method error-prone in respect to contaminations.[9,13]

Extraction by Electrical Energy

ln this method, electrical energy is used in the form of an electric field to accelerate extraction and improve the yield of the extraction. Extraction of scopolamine from the seeds and capsules of Indian thorn apple has been reported by this process.14

Vertical or Turbo Extraction

Vertical or turbo-extraction is used. Here the drug to be extracted is stirred in the menstrum with a high-speed mixer or homogenizer. The shredding and shearing forces break down the drug material to a particle size, which is smaller than that of the material when it is first put in the mixer. The cells become highly disintegrated. The diffusion of extractive substances through the cell membranes is largely replaced by washing out from the destroyed cellular tissues. These result in substantially faster establishment of the maceration equilibrium and hence in a considerable saving of time.[1, 3]

Counter Current Extraction

ln continuous countercurrent extraction, a moving solution, emulsion, suspension or solid mass is extracted by a liquid phase flooring against it. In relative counter current extraction, on the other hand, only one phase (as a rule the extraction solvent) is in motion, the other phase (usually the solid) remains stationary.[18]

Miscellaneous Methods

Following are miscellaneous method used for extraction.

Expression

This method is use to obtain fixed oils from plant material. This involves disruption of the cellular structure by the application of pressure to the material and allows oil to flow out of the material. This method is frequently used for soya oil, sunflower oil and olive oil. The rupture in the cell kernel causes the elution of oil in this method.[5, 9, 13]

Pervaporation- This method is currently being developed and its success will depend on the generation of new membranes which show selective binding for particular chemical groups. Hydrophilic membranes may be used to remove polar materials, including water from organic solvents and hydrophobic membranes can be used to remove organic compounds from an aqueous phase. This method has been used to remove aroma compounds from fruit juices. [12]

Sublimation

ln this process some substances, change from solid to gas or vice versa without passing through a liquid state on heating or cooling. This method can be used to obtain the substance from dried plant material or a dry crude extract. Caffeine of high purity can be obtained by this method from dry tea leaves.

Extraction of alkaloid, sesquiterpene lactone and cardiac glycoside, flavonoids, other polyphenols, sterols, saponins, carbohydrate

Solvent extraction is the most popular method of extraction. The main groups of compound to be considered are fixed oils, fats and waxes, volatile or essential oils, carotenoids, alkaloids, glycosides, aglycones, phenolic compounds, polysaccharides and proteins. Polarity and pH are two important factors. A general outline of the solvents that would be appropriate for extraction depends on above classes of compounds. The methods given in this

section are general ones based on the common properties of broad classes of phytochemicals.[17]

Alkaloids

All alkaloids contain at least one nitrogen atom and the compound is basic. This means that salt formation can occur in the presence of acid. This fundamental property of alkaloids is used in their extraction and further clean-up. Two methods may be used for alkaloid extraction. One is to basify the plant material using diethylamine or ammonia and extract with an organic solvent. [1, 4]

Carotenoids

Carotenoids are responsible for red, orange and yellow pigments observed in the plant and animal kingdoms. They are generally tetraterpenoid and can be divided into hydrocarbons and oxygenated forms known as xanthophylls. Hydrocarbon tetraterpenoid are less polar and can be extracted into petroleum ether. Xanthophylls are more polar therefore be extracted into ethanol or mixtures of ethanol and less polar solvents such as chloroform.[9, 12]

Glycosides

Glycosides are relatively polar in nature and its polarity depends on both number and type of sugar the structure of the aglycone. Most glycosides can be extracted with polar solvents such as acetone, ethanol, methanol, water or mixtures of these. However, cardiac glycosides have bulky steroidal aglycone, which shows appreciable solubility in chloroform. When extracting into water, and sometime enzymatic breakdown also possible. This will not occur if boiling water is used or if significant proportions of alcohol or ammonium sulphate are added to the extract. In some cases, it may be the aglycone rather than the glycoside that is to be extracted, and this requires hydrolytic separation of the aglycone and sugar before or after extraction.[3, 7]

Phenolic Compounds

These can exist as free phenols or in glycosidic form. Due to the multiplicity of hydroxyl functions, phenols tend to be relatively polar and dissolve in aqueous alcohols. As they are weak acids, they may also be extracted or partitioned into aqueous alkali as phenolate salts. A problem encountered with phenolic compounds is that they can undergo extensive polymerization a reaction by the action of polyphenol oxidizes. This reaction will responsible for the development of brown coloration in damaged plant material when exposed to the air and in certain extracts. The polymerization reaction is catalyzed by acid.[14,17]

Proteins

Due to the presence of free carboxylic, amino and phenolic groups on amino acid side- chains in proteins, most can be ionized at high or low pH values. The pH at which no net charge is carried is known as the isoelectric point and this will vary with each protein depending on the constituent amino acids. At pH values above the isoelectric point, the protein carries a net negative charge, and the pH values below the isoelectric point, a net positive charge is carried. As a result of this, most proteins can be extracted with water, buffers, dilute acid or base or simple salt solutions. However, more lipophilic proteins require the use of 70-80% alcohol. Selective precipitation of groups of proteins in a crude protein extract can be achieved by gradual addition of acetone, ethanol or ammonium sulphate. Conversely, for proteins with a greater solubility in salt solutions that water, e.g. globulins, a crude protein mixture can be extracted in 10o/o sodium chloride solution and the globulins precipitates by addition of water. For prolamines, extraction in 70-80% alcohol can be followed by precipitation by dilution with water. The precipitation step can be followed by resolubilization and further separation using ultra filtration, gel filtration, ion-exchange chromatography or electrophoresis.[1, 8, 10, 13]

Polysaccharides

Polysaccharides are polymers of sugars or sugar derivatives. Generally, there are three types of sugar polymers - those that are completely water soluble, those that partially dissolve in water and swell to form gels and lastly, those that are water insoluble. Polysaccharides that totally or partially dissolve in water can be extracted using cold or warm water.

Volatile Oils

Volatile oils are the odorous principals found in various plant parts. Because they evaporate in air at ordinary temperatures, they are called volatile oils, ethereal oils or essential oils.[1]

Fractionation, Purification and Isolation

While different techniques have evolved for the extraction of the phytoconstituents so as to obtain crude extracts (complex mixtures), fractions (simpler

mixtures) and isolated (pure) components from natural sources. These are usually then subjected to a number of further analytical investigations in order to obtain more information on the properties of their constituent substances. Broadly, the nature of further investigations after extraction of a crude drug is of three types:

1. Qualitative chemical analysis - determination of the nature of the constituents of a mixture or the structure of an isolated compound.
2. Quantitative chemical analysis - determination of the purity of an isolated substance or the concentration of a single substance or group of substances in a mixture
3. Bioassays - determination of the biological or pharmacological activity of substances and the dose range over which they exert their effects.

The amount of fraction available is possibly the most important factor in making decisions about its future treatment and analysis. Investigative methods can be either non-destructive or destructive. Non-destructive methods mean that the sample can be recovered and used for other tests. Some physico-chemical procedures, e.g. chemical tests, analytical chromatography, mass spectrometry and all biological testing procedures are destructive i.e. they ule up the sample and generally it cannot be recovered. Another important factor is that th6 different types of analysis require the sample to be present in different types of medium. lt is always desirable to supply the fractions as solids, which can be weighed accurately and reconstituted in the appropriate solvents. The solid forms are usually produced from solutions by evaporation under reduced pressure to minimize decomposition or, in the case of aqueous solutions, by freeze-drying, sometimes called lyophilization.[13,16,17]

Separation/ Fractionation/ Isolation

1) Sublimation
2) Distillation
3) Evaporation
4) Fractional liberation
5) Fractional crystallization
6) Fractional distillation
7) Chromatography

Fractionation

All separation processes involve the division of a mixture into a number of discrete fractions. These fractional may be physically discrete fraction.

In which successive partition into diethyl ether, chloroform and ethyl acetate will often afford in turn flavonones and flavonols, methoxylated flavonoids and flavonoid monoglycosides. Di- and polyglycosylated flavonoids will remain in the residual aqueous phase. Saponins are water soluble compounds, and the crude saponin fractions are water soluble compounds, and the crude saponin fractions may be obtained. The alkaloids are basic compounds may be extracted either into aqueous acid solution after removal of neutral impurities with an organic solvent, or by treating the groups wet plant material with an alkaline substance such as CaCO3 powder and extracting with diethyl ether after standing overnight. Specific literature should be consulted for specific plant extraction problems.[10,15]

Sublimation

Sublimation is used for isolation of caffeine from tea, for its purification of materials present in a crude extract and for separation of camphor.[17]

Distillation

Steam distillation is much used to isolate volatile oils and hydrocyanic acid form plant material. The TAS oven is used for steam distillation on a semi micro scale for the direct transfer of volatile materials from a powdered drug to thin layer plate.[16]

Fractional liberation

Some compounds are separated by fractional liberation from a mixture. A mixture of alkaloid salts in aqueous solution, when treated with aliquots of alkali, will give first the weakest base in the free salt followed by base liberation in ascending order of basicity. If the mixture is shaken with an organic solvent after each addition, then a fractionated series of base will be obtained. A similar procedure is used for organic acids soluble in water immiscible solvents. In this case, starting with a mixture of the acid salts, it is possible to fractionally liberate the acids by addition of mineral acids. Sodium salts of acids on treatment with dilute HCl yield free organic acids.[10, 18]

Fractional crystallization

The method is used on the difference in solubility of the components of a mixture in a particular solvent. Frequently, derivatives of the particular components are used (picrates of alkaloids, osazones of sugars)[12, 17]

Chromatography

Chromatography represents a group of techniques for separation of compounds of mixtures by their

continuous distribution between two phases, one of which is moving past the other.

Paper Chromatography

Paper is used as stationary phase and mobile phase is used for separation of components.

Solvent Recycling

For both economical and ecological reasons the recovery of solvent is important. Use of single solvent is much better rather than azeotropic mixture which is difficult to separate into individual components. Complex distillation procedure is used for solvent recovery. An adequate care must be taken in disposing of solvents in a way that causes least damage to the environment. Most scientific establishments have standard procedures for the disposal of organic solvents.[13, 17, 18]

Treatment of Drug Residue after Extraction

Further treatment of the drug residue is very essential and more important in the context of extraction of herbal drugs. This is necessary for several reasons as follows:

1. The drug residue contains considerable quantities of absorbed solution with valuable extractive substances
2. The solvents used should be recovered
3. There will be further uses of the drug residue, which necessitate removal of the solvents which must not be equated with recovery of the solvents)

During extraction, the drug material, depending on its nature and the species of plant from which it is obtained, absorbs varying quantities of solvent and hence swells up to varying degrees. The drug residue has a species-dependent retention capacity for solvents, which is known as the 'absorption capacity'. The solvent can, in principle, be removed in two ways,

(1) Expression of the drug residue

(2) Expulsion of the solvent by warming with or without pressure reduction.[11, 13, 15]

Conclusion

Natural product drug discovery program a long term capital-intensive program. Several extraction, fractionation/ separation and isolation methods are developed after which isolation of the active moiety and their chemical examination is performed. Microwave extraction, sonication, lyophilization, spray drying and vacuum drying have also been employed with good results. Generally the extraction is based on pharmacological activity rather than chemical nature of compounds. Further studies are carried out after the isolation of the active moiety to confirm the compound. The phytochemical investigation of a plant involves the selection, collection, identification and authentication, extraction of the plant material (first fractionation), fractionation/separation (second fractionation) and isolation (third fractionation) of the constituents, characterization of the isolated compounds and investigation of the biosynthetic pathways of particular compound, quantitative evaluations and pharmacological activities.

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Table 1: Term and Explanation.

S.No	Terms	Explanation
1	Menstrum	Solvent or mixture of solvent used for extraction
2	Rinsing	Dissolution of extracted components out of lysed cell
3	Lixiviation or leaching	Extraction of components using water
4	Miscella	Filtrate or solution containing extracted components

Table 2: Nature of Constituents or Secondary Metabolites

Leaf			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Peppermint (*Mentha spicata*)	*l-menthol, I carvone*	Flavouring agent
2	Senna (*Cassia angustifolia*)	Anthrquinone glycoside Sennoside A ans Sennoside B	Purgative
3	Digitalis (*Digitalis lanata*)	Digoxin, Digitoxin	Cardiotonic
Seed			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Nux vomica (*Strychnus nux-vomica*)	Strychnine, Brucine	Stimulant to CNS and respiractory system, Increase B.P.
2	Strophanthus combii, S. gratus	Stropanthidin	Cardiotonic
3	Isabgol (Plantago ovata)	Mucilage	Laxative
Bark			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Cinchona (*Cinchona zeylanicum, Cinchona succirubra, Cinchona officinalis, Cinchona letgerinia*)	Quinine, Quinidine	Antimalarial, Anti-arrhythmic, Antipyretic
2	Kurchi (*Hollharena dysentrica*)	Steroidal alkaloid Conessin	Antiprotozal
3	Cinnamon (Cinnnamomum zeylanicum)	Eugenol	Carminative, Antiseptic

Wood			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Quassia (*Picrisma excelsa*)	Quassin	Bitters, Stomachic
2	Sandle wood (Santalum album)	Isomeric sesquiterpent alcohol, α-santalol, β-santalol	Micturation
Root			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Ashwagandha (*Withania somnifera*)	Withanine, Somniferine, Withanolide (Steroid)	Sedative, antirheumatic
2	Ipecac (*Cephalis ipecacuanha*)	Emetin, cephalin	Expectorant, Emetic, Antiamoebic
3	Aconite (Aconite naphellus)	Aconitine, Neopelline	Rheumatic and sciatica treatment
Rhizomes			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Turmeric (*Curcuma longa*)	Curcumin	Anticancer, Hepatoprotective
2	Ginger (*Gingiber officinalis*)	α-zingiberenr	Carminative, stimulant
3	Podophyllum (*Podophyllum hexandrum*)	Podophylotoxin, podophyllin	Cytotoxic, Veneral and other warts .
Flowers			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Saffron (*Crocus sativus*)	Crocin, crocetin, picrocrocin	Antispasmodic, Stimulant, emmenagogue
2	Pyrethrum	Insect repellant	
3	Clove (*Eugenia caryophyllata*)	Eugenol	Local ansthesia, Carminative, antiseptic
Fruit			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Corriandar (Corriande sativum)	Coriandrol geraniol	Flavour, carminative, stimulant
2	Colocynth (Citrullus Colocynthis)	α-elaterin, colocynthin	Purgative, carminative
3	Fennel (*Foenoculum vulgaris*)	Fenchone, anethole	Carminative, stimulant, expectorant
Entire plant			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Ephedra (*Ephedra gerardiana*)	Ephedrine, Pseudoephedrine	Hat fever treatment, Sympathomimetic
2	Ergot (Claviceps purpurea)	Ergometrine, Ergotamine	Oxyctocic, Migrane treatment
3	Belladonna (*Atropa belladona*)	l-hyoscyamine, atropine	Antispasmodic, anticholinergic
Dried Latics			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Opium (*Papaver somniferum*)	Narcotine, papaverine	Narcotic analgesic, diarrhoea
2	Dried latices	Papain (Carica papaya)	Proteolytic enzymes	Meat tenderiser
Resins			
S.No.	**Plant (Botanical Name)**	**Chemical constituents**	**Uses**
1	Balsum of tolu (Myroxylon balsamum)	Cinnamic acid, benzoic acid, benzyl benzoate, benzyl cinnamate	Expectorant, Antiseptic
2	Myrrh (Commiphora molomol)	α-commiphoric acid, β-commiphoric acid, γ-commiphoric acid	Stimulant, Antiseptic
3	Asafoetida (Ferula foetida)	Ferulic acid, Umbellic acid	Nervien stimulat, carminative

Table 3: Dielectric constant of solvent[11]

S.No.	Solvent (increasing eluting power)	Dielectric constant	Type of solvent
1.	Cyclohexane	18.5	Non polar
2.	Ether (Ethanol free)	3.8	Polar protic
3.	Acetone	20.7	Polar aprotic
4.	Benzene	2.3	Non polar
5.	Toluene	2.38	Non polar
6.	Chloroform	4.8	Non polar
7.	Acetonitrile	37.5	Polar aprotic
8.	Water	80	Polar

Table 4: Solute and solvent.

S.No	Solute	Solvent
1	Oils and fats	Carbon tetrachloride, benzene and mineral oil
2	Alkaloidal bases	Non-polar solvents
3	Fatty acids	Non-polar solvents

Table 5: solvent miscibility.

S.No	Semi-polar solvent	Improved miscibility
1	Acetone	Ether in water
2	Propylene glycol	Water in peppermint oil

Table 6: Binary and Ternary Azeotropic Mixtures.

Binary Azeotropic Mixture			
S.No	**Solvent Mixture**	**Ratio**	**Boiling point**
1	Methanol:n-hexane	27:73	50
2	n-hexane:chloroform	28:72	60
3	Ethanol:Water	95.57:4.43	78.15
Ternary Azeotropic Mixture			
S.No	**Solvent Mixture**	**Ratio**	**Boiling point**
1	Ethanol:carbon tetrachloride:Water	23:57.6:19.4	61.8
2	n-propanol:cyclohexane:Water	18:54.8:26.9	65.4
3	n-propanol:carbon tetrachloride:Water	8.9:62.8:29.3	68.5

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