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PHYTOCHEMICALS: EXTRACTION, ISOLATION METHODS,IDENTIFICATION AND THERAPEUTIC USES: A REVIEW

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ABSTRACT

Phytoconstituents refers to the compounds which are chemical in nature that eventuate in plants. These compounds are non-nutrient compounds which are bioactive and helps in preserving the plants from various kinds of infections or other kinds of pathogens or microorganisms whereas some are creditworthy for the organoleptic characteristics. These compounds are synthesized within the plants from end to end of primary and secondary metabolic pathways. Various active components are discovered and are included in various categories. These active components include alkaloids, glycosides, volatile oils, phenolics, saponins and tannins etc.. Extraction of these compounds can be done by various methods. For the purpose of extraction that is to extract active components from the plants, high accuracy is needed along with different types of solvents with differing polarities. Various studies done on these experiments tells us that high polarity solvents have high effects. Various extraction techniques are maceration, infusion, decoction, percolation, digestion, soxhlet extraction, aqueous alcoholic extraction by fermentation, supercritical fluid extraction, etc.. These types of techniques are very useful in extraction process. The active components are isolated and purified for requirements. These are done by various chromatographic techniques such as Thin layer chromatography, gel permeation chromatography, etc.. The diverse form of processes for the active components are reviewed in this paper.

Keywords: Phytochemicals, extraction, polarity, TLC, SCF, bioactive compounds.

Introduction

Various drug discoveries took place in the past years which were basically obtained from plants and their metabolites. Drug discovery from the natural sources required a deep knowledge about their pharmacological use, chemistry, biology and toxicology as well. Plants are used as medicine from ancient times (Sasidharan *et al.,* 2011) Nowadays the use of medicinal plants is done by extracting the active components. Phytochemicals are the biologically active compounds present in the plants. The phytochemicals are the sources of direct medicinal agents and are obtained from seed coat, flowers, roots, leaves, barks, seed and pulp of the plants. The secondary metabolites from the plants are extracted by various solvent systems using different extraction techniques. They are basically the derivatives of the primary metabolites and are not responsible for the growth and development directly. There are various techniques for the extraction of such metabolites. As we have already discussed that Phytochemicals are the elements which transpire anticipatingly from plants. Some at the helm of tone and various organoleptic possessions. Active constituents present in there are very useful. They are not only used for medicinal use but also for some phytoconstituents like volatile oils are used as perfumery and some even as food flavors and preservatives. These may be physiological possessions but not organic elements (Altemimi *et al.,* 2017). There are various number of automations available for the extraction of active compound from either of the aromatic plant or medicinal plant. They also help in the treatment of various diseases. The treatment of various diseases utilizing these phytochemicals is more effective than the synthetically prepared medicines because they have less risk of side effects. This natural medicinal source is not only used in the developing countries but has also proved to be effective in the developed countries. The secondary metabolites are used for medicinal purpose. Gymnosperm is one of the compounds having secondary metabolites of medicinal purpose. It is a source of taxol which is used as a essential oil, diterpenoids, lignans, steroids, sterols and bioflavonoids. It is also used to treat disorders of the digestive, respiratory, nervous and skeletal systems. In pharmacologically it is used for antiepileptic, anti-inflammatory, anticancer, antipyretic, analgesic, immunomodulatory and antimicrobial activities. There is various disease like diabetes, asthma, cancer, inflammatory disease, etc. which have been cured by the phytoconstituents. Phytoconstituents amalgamated in plants from far side of primary and secondary metabolism routes. Secondary metabolites which are generated in plants are used for transmission as signal constituents to captivate discrete pollinating agents counting insects, birds, etc. Even fruits and vegetables can be directly consumed to obtain various health benefits and along with medicinal properties they also have

high nutritional value (Varma, 2016). As stated by world health organization, above 80% population is subservient on medicinal plants to sustain their health and to cure their affliction. It is now accepted and have confidence in phytoconstituents procured from the medicinal plant's molecules in the modern medicines. Extraction can be defined as the process in which medicinally active phytoconstituents are separated using selective solvents by applying some standard procedures. The nature of the phytoconstituents helps in the selection of solvent for the extraction purpose. The commonly used extraction methods include maceration, infusion, percolation, decoction, soxhlet extraction, superficial fluid extraction, microwave assisted extraction, etc. The complex mixture of various plant metabolites like glycosides, alkaloids, phenolics, terpenoids, etc. are present in the initial crude drug extract obtained by means of extraction (Sasidharan *et al*., 2011). Before the process of extraction, the plant parts which are rich in bioactive compounds are kept separately for drying. These plant parts may include roots, stem, bark, leaves, etc. (Azwanida, 2015). The phytoconstituents are extracted on the nature of the polarity strength of the solvent which is used in process of extraction .The polarity strength of the solvents can be divided into three basic categories which include nonpolar, medium polar and polar. Soxhlet extraction, maceration, steam distillation and hydro distillation are some of the most commonly preferred extraction techniques because they are simple and convenient (Majekodunmi, 2015). There are various properties of the extraction solvent which influence the extraction efficiency. Such properties may include temperature, solvent ratio, duration of extraction etc. (Zhang, 2018). Plant metabolites for curable impetus is earning admiration in today's world. The virtual momentous gradation of metabolites is extraction and isolation of constituent of interest. Now a days we can designate two unit of extraction techniques known as conventional technology and new or green technology. One of which is cheaper and requires high amount of solvent and acquires long duration of time whereas the other one is costly with less duration of time respectively. Subsequently post the work of extraction of the secondary metabolites purification and isolation is done via using chromatographic and non-chromatographic techniques (Kaur, 2018).

Types of Phytoconstituents

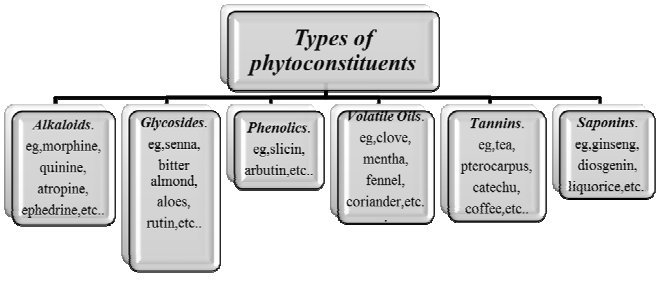
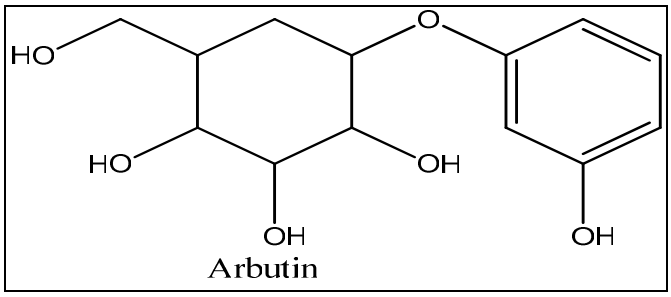


Fig. 1: Classification of Phytoconstituents

Phenolics

These compounds are the major group of phytochemicals which are diverse in nature including flavonoids, polyphenolic amides. Almost every plant consists of phenolic compounds but they have slight difference in their bioavailability like Arbutin. The biosynthesis of phenolics is a complex process (Milgate *et al.*, 1995).



Isolation of phenolic compounds

These can be isolated by different chromatographic techniques:

1. High performance liquid chromatography
2. Column chromatography

Identification Test

Ferric Chloride Test-On addition of aqueous ferric chloride to the compounds with phenol group ,violet, green, blue, purple or red brown colour is obtained.

Pharmacological Use

Natural phenolic compounds are generally used to treat and prevent the severe disease like antarthritic, cancer and wound healing etc. (Essays, 2018).

Alkaloids

Alkaloids are the organic products of synthetic or natural origin which mainly contain a basic nitrogen atom which is heterocyclic in nature and have marked physiological actions on the body of humans and animals e.g. quinine, morphine, ephedrine, atropine etc.

Carl Friedrich Wilhelm Meissner, a German chemist introduced the name alkaloids.

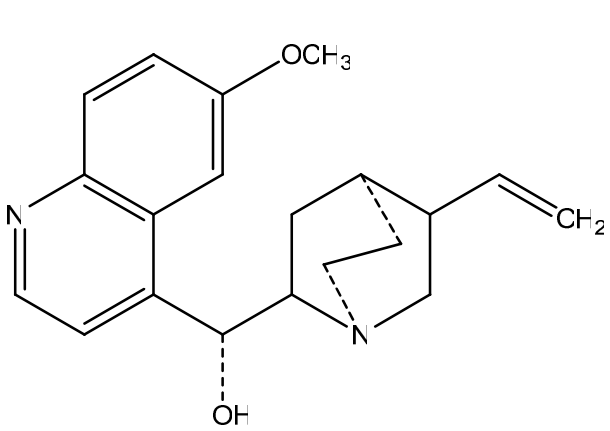
These alkaloids can be classified into various categories:

1. True alkaloids - They are mainly originated from the amino acids and consist of a nitrogen atom in the heterocycle. These may include the following examples like nicotine, atropine, and morphine. Few exceptions of this class: which along with the nitrogen atom in the heterocycle also contain terpene (e.g.Evonine) or the peptide fragments (e.g. Ergotamine).

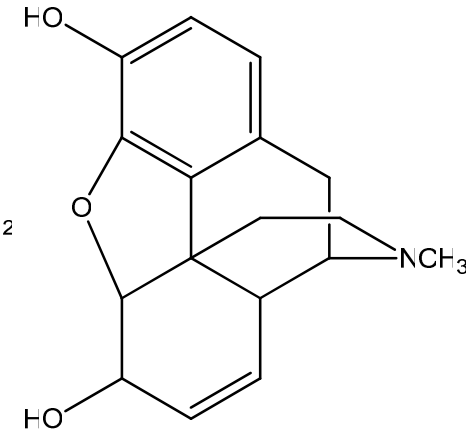
2. Protoalkaloids- They also originate from amino acids and contain nitrogen atom outside the heterocycle. Their examples are mescaline, ephedrine and adrenaline.

3. Polyamine alkaloids -They are referred to as the derivatives of spermidine, putrescin and spermine.

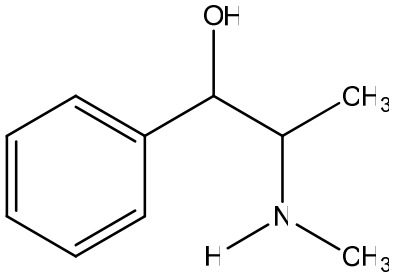
4. Pseudoalkaloids – These are generally not derived from amino acids but from acyl CoA units. The examples may include Conestine, caffeine (Aniszewski, 2007), (Cordell *et al.,* 2001).



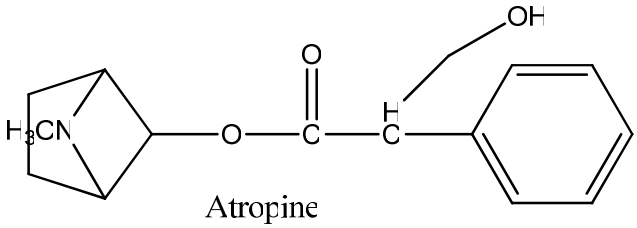
Quinine



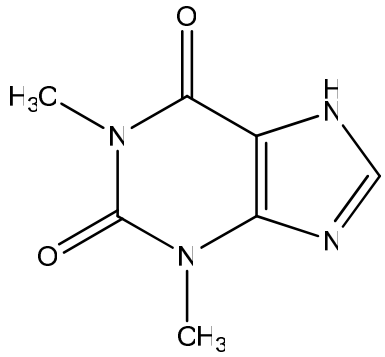
Morphine



Ehedrine



Atropine



Caffeine

Method of isolation

Some widely accepted methods used for the extraction of alkaloids are:

1. Soxhlet extraction process
2. Kippenberger's process
3. Stas Otto process (Kaur *et al.,* 2015).

Identification Test

1. Cream coloured precipitates are formed using Mayer's reagent (potassium mercuric iodide solution).
2. Reddish brown precipitates formed using Dragendorff's reagent (nitic acid and solution of potassium bismuth iodide).
3. Reddish brown precipitates formed using Wagner's reagent (solution of iodine potassium iodide).
4. Yellow coloured precipitates are formed with Hager's reagent (picric acid solution which is saturated).

Pharmacological Uses

1. Antimalarial activity of quinine
2. Vasodialatory effect of vincanine
3. Antiarrhythmic effect of quinidine
4. Antibacterial effect
5. Analgesic effect of morphine
6. Anticancer
7. Antiasthamatic (Zdunić*et al.,* 2011)

Glycosides

A compound is referred to as glycoside if carbohydrate and noncarbohydrate part is present in one single molecule. A carbohydrate and a noncarbohydrate portion is attached with the acetal linkage. There are two components present in a a glycoside: glycone means the sugar component and aglycone means the no sugar component.

They can be classified as:

1. Anthraquinone glycosides
2. Cardiac glycosides
3. Saponin glycosides
4. Alcoholic glycosides
5. Phenol glycosides
6. Coumarin glycosides

7. Isothiocyanate glycosides

8. Cyanogenetic glycosides

9. Flavonol

10. Iridoid(Chinthapatla).

Method of Isolation

Glycosides can be obtained from crude extract by using various processes like fractional solubility, fractional crystallization and chromatographic methods such as thin layer chromatography, column chromatography.

Identification Test

1. Borntragger's test- Aqueous solution of drug is taken. Boiled and filtered after adding 1 ml sulphuric acid. Equal volume of chloroform or ether is added to the filtrate. The organic layer forms. It is then separated & the solution of ammonia is added to it.Pink or red colour of ammonia layer shows the presence of anthraquinone glycosides.

2. Modified born tragger's test - Aqueous solution of drug is taken. To this sulphuric acid along with ferric chloride is added and boiled gently. Filtered and added chloroform or ether to the filtrate. The organic layer forms. It is then separated & the solution of ammonia is added to it. Rose pink or red colour of ammonia layer shows the presence of C-glycoside.

3. Keller-killiani test - Chloroform is added to the powdered drug which is boiled gentally and filtered. Glacial acetic acid along with 0.2 ml of 5% ferric chloride is added to the filtrate. Further there is an addition of 2 ml sulphuric acid. There is a formation of red - brown colour at junction of liquid changes to bluish green.

4. Legal's test- Alcoholic extract of drug along with 1ml of sodium nitroprusside & pyridine results in pink to red coloration.

5. Baljet test- Powdered drug and 2ml sodium picrate solution gives yellow to orange colour.

6. Kedde's test- To the purified 2-3 ml chloroform fraction of extract, add 1 ml of Kedde's reagent (equal volumes of 2% 3,5-dinitrobenzoic acid in alcohol and 10 % NaOH)→ purple-violet color

7. Sodium picrate test- Aqueous solution of drug and dilute sulphuric acid are taken in a flask. A filter paper treated with sodium picrate is suspended. Filter paper turns brick red.

8. Froth test- Stable forth is formed when the powdered drug and water are shaken well.

9. Alkali test: Blue green fluorescence colour confirms the presence of coumarin glycosides when ammonia or caustic soda is added to alcoholic extract of the drug.

10. Shinoda test- Few drops of hydrochloric acid along with 0.5 g magnesium turnings is added to alcoholic extract. Pink colour is obtained.

11. Lead acetate test- Yellow precipitates are formed on addition of lead acetate solution to the powdered drug.

Pharmacological Uses

Used as laxative, carminative, purgative, skin cosmetics, soothing and healing properties, etc. (Kaur *et al.,* 2015; Zdunić *et al.,* 2011)

Tannins

Tannins are defined as the complex organic compounds which occur naturally and possess nitrogen free polyphenols of high molecular weight. Their molecular weight ranges from 500 to 3000 and 20,000.They can be classified into the following categories:

1. Hydrolysable tannins – The tannins which can be hydrolyzed by enzymes or acids and produce gallic and ellagic acid are called hydrolysable tannins. These tannins produce blue/black colour when they are treated with ferric chloride.

2. Condensed tannins –The tannins which do not undergo any hydrolysis are called condensed or non-hydrolysable tannins. They produce catechol when they go through dry distillation process therefore, they are also known as catechol tannins. These tannins get decomposed to phlobaphenes when they are treated with various acids or enzymes (Minocha *et al.,* 2015).

3. Pseudo Tannins – The phenolic compounds possessing low molecular weight are called pseudo tannins. The generally do no pass the goldbeater's skin test.

Identification test

1. Gelatin test: Formation of white buff coloured precipitates marks the presence of tannins when 1% of gelatin solution &10% solution of NaCl is added to aqueous drug solution.

2. Goldbeater's skin test: When some portion of gold beater skin is put in 20% HCl acid, rinsed by water & kept in the solution of drug for approx.. 5 min, it gives brown or black colour on the skin which confirms the presence of tannins.

3. Phenazone test: Bulky yellow precipitates form when 2% phenazone solution is added to aqueous solution of a drug.

4. Match stick test (Catechin test): Matchstick wood turns from pink to red when it is dipped in aqueous extract of the plant, then dried, then moistening it with conc. HCl and eventually warming it near the flame.

5. Ferric chloride test: Brownish green colour appears when 1% ferric chloride is added to tge alcoholic extract of the drug.

6. Vanillin-hydrochloric acid test: Red or pink colour appears when 1g vanillin, 10 ml alcohol, 10 ml concentrated hydrochloric acid are added to the drug.

Pharmacological Uses

Tannins are used as strong astringent, mild antiseptic, in treatment of diarrhoea, haemostatic, burns, scars of the skin (Zdunić *et al.,* 2011).

Volatile Oils

The oils which at ordinary temperature gets evaporated when exposed to air. They are responsible for the odour and therefore be referred to as essential oils. They can be originated from plants and animals e.g. eugenol, camphor, carvacrol and thujone etc. They can be classified on the basis of following:

1. Essential oil components are categorized into terpenoids and non-terpenoids.

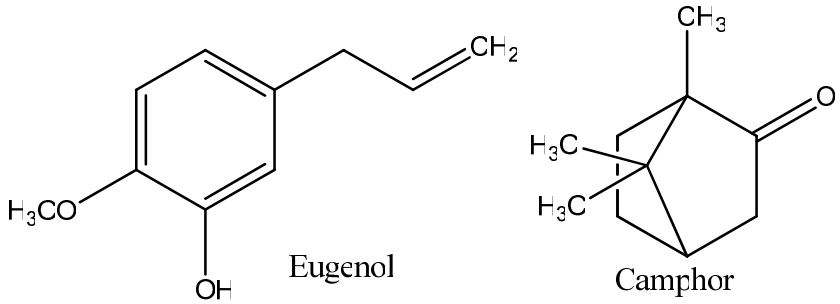
1. Terpenoids: They are the mixtures of the following –

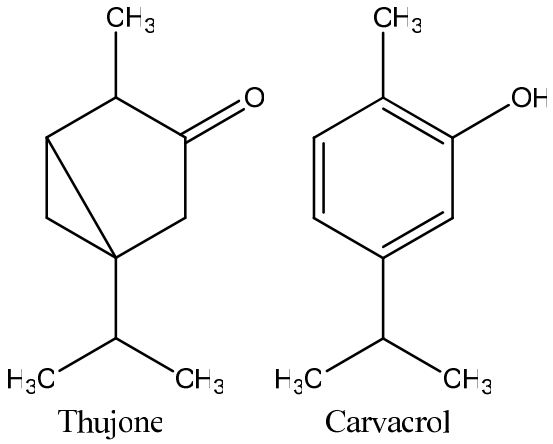
    1. Hemiterpenes
    2. Monoterpenes
    3. Sesquiterpenes
    4. Diterpenes
    5. Triterpenes
    6. Tetraterpenes
    7. Polyterpenes

2. **Non terpenoids:** This group consist of Sulphur containing substances, aromatic substances, short chain aliphatic substances and nitrogenated substances (Varma, 2016).

B) On the basis of functional groups:

1. Hydrocarbon
2. Alcohol
3. Aldehyde
4. Ester
5. Ketone
6. Phenol
7. Ether
8. Oxide





Method of Isolation

There are different techniques by which volatile oils can be extracted from plants. Some of these include solvent extraction, maceration, water distillation, cold press extraction, steam distillation.

Identification Test

1. Red colour globules are formed when alcoholic sudan red 3 is added to this section of a drug.
2. Red coulour globules are formed when a drop a tincture alkane is added to this section of a drug.
3. Place a drop of voaltile oil on filter paper,when observed after 5 min ; no translucent spot is there.

Pharmacological Uses

1. Improves local circulation
2. As carminative
3. Local anaesthetic
4. Thymol uses in gargles and mouthwashes
5. Used in aromatherapy, etc.(Kaur, *et al.,* 2015).

Saponins

Saponins are the glycosides which generally have the characteristic of forming foam. This foam is formed by the combination of the aglycone part (sapogenin) which is hydrophobic in nature and the glycone (sugar) part which is hydrophilic in nature. Various plants like peas, beans and various other herbs are the major sources of saponins. *Yucca schidigera* and *Quillaja saponaria* are the two species from where commercial saponin can be extracted. They also have the detergent properties and are used in cosmetic creams, shampoos, etc. (Sindhu *et al.,* 2011).

Isolation

1. Soxhlet extraction
2. Thin layer chromatography

Identification test

1. Test for Haemolysis 0.2 ml solution of saponin is mixed with 0.2 ml blood & further centrifugated. Note the supernatant and compare it with the control tube which contains 0.2 ml of 10% blood in normal saline which is then diluted with 0.2 ml normal saline.

2. Test for Froth formation: A froth (foam) formation takes place when 2 ml of drug solution in water in test tube is shaken (Essays,2018).

Pharmacological Uses

1. Reduces cholesterol
2. Reduce bone loss
3. Boosts the immunity
4. Antioxidant
5. Reduces the risk of cancer (Zdunic *et al.,* 2011).

Commonly Used Methods of Extraction

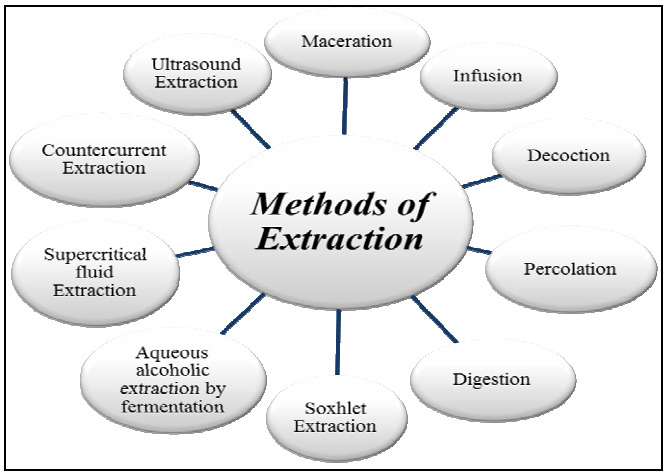


Fig. 2: Methods of extraction

1. Maceration- Maceration is simple method for extraction of crude drug for further medicinal preparation.It is a low cost technique to get phytochemicals from plant material at room temperature. In this, plant material in the form of powder is added into a closed container with solvent for 7 days without continuous stirring. After 7 days, solvent like alcohol, water, oil etc. is removed from the powder by pressing the powder material (Varma, 2016). Maceration process is divided into three types-

1. Modified maceration- The unorganized drugs like resins, gums are extracted by this method. Soluble material is dissolved into a solvent and transferred into a conical flask for 2-7 days, then filtered the product and filtrate is collected.

2. Multiple maceration- It is same as the modified maceration but in this solvent is divided into parts (two for double and three for triple). Double maceration- volume of solvent/2 + first maceration = volume taken by the drug. triple maceration- volume of solvent for firsttmaceration/3 + volume taken by the drug. double maceration- volume of solvent/2 + first maceration = volume taken by the drug.

3. Vacuum extraction- This method is done in a much shorter time. In this, vessel is connected with a vacuum pump.

Merits: Easy to handle, Low investment, Automated system.

Demerits: Not suitable of heat sensitive pigment, Long extraction time, large solvent consumption (Yalavarthi, 2013).

2. Infusion: Infusion is a very simple method in which preparations are prepared from leaves. In this powdered drug is soaked in either cold or boiling water for short period of time (Sindhu *et al.,* 2011). It is used for those plants which are dissolved easily and release their active ingredients.

Merits: Less time needed for infusion process, Thermolabile constituents may also be extracted.

Demerits: There is a need of more time as compared to soxhlation, need of more solvent, Need of trained person (Majekodunmi, 2015).

3. Decoction: It is a process of extraction, in which we use only plant products, plant materials or parts of plants. So, the decoction technique is basically used for extraction of plant. In this, plant materials like leaves, stem, flowers etc. are boiled in water for some time that is only dependent on the part of the plant or type of plant. For leaves, flowers, stems etc., less time for boiling is needed that's about 15-20minutes or less but other hard parts of plants require more time for boiling that's about 1hour or more. After boiling, cool it and add some amount of cold water which is required for the decoction process and filtration is done through cloth in order to remove the water or any liquid medium and then stored it (not more than 24 hours).

Merits: Produce liquids with differing chemical properties, more soluble chemicals may produce (Sindhu *et al.,* 2013).

Demerits: Water is not a good solvent for many of the active components in herbs.

4. Percolation- Percolation is a continuous process of extraction which depends on the diffusion that's already a another method of extraction. In diffusion, liquid moves from higher concentration to lower concentration (Zhang *et al.,* 2018). In percolation, the solvent also moves or flow from higher concentration to lower concentration until it absorbs constituents from the container through bottom filtered. It has an advantage that it shows continuous gravity for flow of solvent that is fresh or unsaturated and the soluble constituents are removed by the solvent. Percolation process is continued at room temperature without agitation, until its all the constituents are extracted.

Merits: Short time and more complete extraction.

- Suitable method for potent and costly drugs.

Demerits: There is a need of trained persons.

- There is need of special attention on the particle size of the material and whole Process (Anonymous,2011).

5. Digestion: Digestion is a mostly used method or easy method that's used for that parts of plant which are poorly soluble in water or other liquid medium. Liquid form of digestion isused by heating because the temperature does not show any effect on the active ingredients of plant or liquid medium in digestion. After heating, it is cooled and filtered.

Merits: Time saving process.

- Acid consumption is lower.

Demerits- Digestion system have only one demerit, namely explosion and cracking of digestion tubes due to simultaneously buildup of pressure along with increase of temperature(Mashfiya *et al.,* 2016).

6. Soxhlet extraction - It is mostly used method of extraction in labs. It is also known as the continuous hot percolation process that's used for insoluble constituents. In this, fixed oils from seeds and alkaloids are extracted by

continuous hot percolation process using different solvents like pet. ether, chloroform, benzene, ethanol and water in order of increasing polarity. The drug is packed into the thimble, which is made from the filter paper and it is inserted into the central tube of extractor. Firstly, non-polar solvent is placed in the flask and it starts boiling. During boiling, solvent produce vapours. Vapours passes from the larger right hand tube into the upper part of the drug and then to the condenser where it condenses and drops come back on the drug. This process is continued till the whole drug is extracted.

Merits: Easy to handle.

- Automated system.

Demerits: Not suitable for heat sensitive pigments.

- Long extraction method.
- Large solvent consumption (Ngaha *et al.,* 2017), (Daswani *et al.,* 2011).

7. Aqueousalcoholic extraction by fermentation

This method involves soaking of many types of drugs such as the crude drugs, in the form of the powder or a decoction for a described period of time, during which it undergoes fermentation and generates alcohol. So this method is used for extraction of the crude drugs contained in the plant materials.

This method is mainly used for the extraction of the crude drugs which are obtained from the plant materials. Some example of this preparations are kanakasava and kapurasava (Costa *et al.,* 2013).

8. Supercritical fluid extraction

Superficial fluid extraction is a technology of separating the one component from another component using superficial fluid as that of the extracting solvents. It is most widely used technique for the extraction. This extraction process is the solubility of the target compound in the selected solvent and solute. Supercritical fluid extraction comes out as a alternative technique for the extraction of bioactive compounds from natural products.

Merits: Filtration is not required.

- It is automated system.
- It is nontoxic.

Demerits: There is a risk of system clogging.

There is also the risk of volatile analyte loses (Tsubaki *et al.,* 2010).

9. Counter Current Extraction

This method is basically used for the extraction of liquid- liquid components. So, it is permitted for the separation of the substances with the different distribution ratios.

In this method, the solvent is present in a large amount and the other one i.e. solute is present in a small amount as compared to the solvents.

The liquid-liquid extraction basically takes place at the same time in all tubes of the apparatus which are used for the extraction as electromechanically.

The separation of the active components between the water and organic solvents basically depends upon the hydrophillic groups which are present in the basic molecule (Baldermann *et al.,* 2008).

10. Ultrasound extraction

Ultrasound extraction is also known as the sono extraction method. This method involves the use of ultrasound with the different frequencies ranging from the 20mhz because they increase the permeability of the cell walls. This process is used in many cases such as the extraction of the natural roots of the plants. It is used for a large scale production due to their high costs. It is most simple method for the disruption of the cell walls (Berthod *et al.,* 2003).

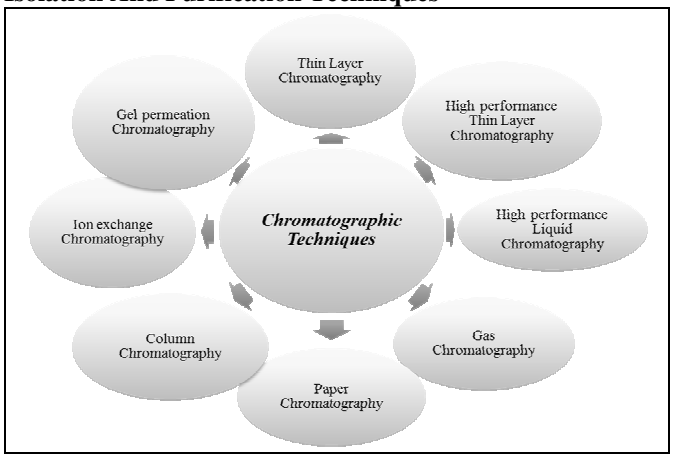
Merits: They consume small amount of fossil energy.

- They have low investment cost.

Demerits: Harmful effect of ultrasound on active constituents.

It is Large solvent volume filtration (Kaubaa *et al.,* 2018).

Isolation And Purification Techniques



1. Thin layer chromatography- Thin layer chromatography (TLC) is used to separate the mixtures. This can be performed on various platforms such as glass, plastic and aluminum foil coated with absorbents. The sample is first applied onto the surface and after that solvent is absorbed on the surface of the plate. This technique may be utilized for monitoring the process and identifying components and to check the purity of the compound. Polar compounds which are more polar has strong interlinking with the motion phase whereas the less polar constituents' spreads to the highest of the surface. If the motion phase is swapped with more polar compound or solvent it would be more useful for dispelling the analytes from silica gel and it would move to the higher (Tyśkiewicz *et al.,* 2018).

Merits: Microlite is the only sample required.

- This is simple method to separate the constituents.
- This technique is sensitive.
- The non-volatile compounds are separated by the TLC method.
- In comparison to other methods, very few equipment's are used.

Demerits: Results obtain from thin layer chromatography are hard to replicate.

- The only constituents which are possible are solubilized components.
- In this technique, no quantitative analysis is being done.

Mainly, it's not an automation technique (Mohammad *et al.,* 2010; Beckett *et al.,*1988; Dreher,1999).

2. High performance thin layer chromatography: This type of chromatography is automation kind of technique which has way better separating economy and sophisticated detection parameters. This technique is also referred to as high pressure thin layer chromatography or also known by either planar chromatography or else know by flatbed chromatography. This technique is measure of analysis and equally desirable for quality and quantity. Partition coefficient or adsorption helps in the separation process (Sethi, 1996).

Merits: Shorter developing time and analysis time.

- Parallel separation of many samples with minimal time requirement.
- Lower amount of mobile phase/solvent consumption.

Demerits: Too costly.

- Maintenance of instrument is difficult.

Specialized test for handling or using is needed (Christophoridou *et al.,* 2005).

3. High Performance liquid chromatography - This type of technique is a certain signifier of column chromatography utilized in biochemical studies for distinct elements, to indicate and qualify the active compounds (Zweig *et al.,* 1973). It is also called a high-pressure liquid chromatography. It basically has a chromatographic column which deemed packed corporeal which is the stationary phase, supplier which transfers the mobile phase through with the chromatographic column. validated sample is being used, therefore hold backwards particularly by chemically or else physically interlinked by the stable phase. Total non-progressive is determined by the nature of the both stationary as well as motion phase. time-period at what the particular analyte moves out from the last of the chromatographic column is referred as retention time. Prevalent solvent utilized includes any sort of miscible consolidation of organic liquid and water.

Example: The basic example is acetonitrile and methanol. Separating is done to check the composition of motion phase during analysis. It moves apart the object of analysis solution i.e. analyte for the present mobile phase.

Merits: Higher resolution and speed of analysis.

- Easy automation of instrument operation and data analysis.
- HPLC columns can be reused without repacking and regeneration.

Demerits: Cost

- Complexity.

Low sensitivity to some compounds (Malviya, *et al.,* 2010; Nikam *et al.,* 2012).

4. *Gas chromatography*- This type of technique is basically used to give a visual display of the amount of the substance of variable segment in the sample. The inspection done by a particular gas chromatograph is referred as gas chromatography (Wang *et al.,* 2003). This technique is basically a kind of technique which is utilized as a analytical science which separate and examine aggravate which can be vaporized without splitting. This technique basically tries to immaculate specific substances or splitting them. In a various situation this technique helps in identifying the compound. This results in pure compound.

This type of chromatography is used as a chemical analytical apparatus for isolating different types of chemicals in specific sample solutions. A gas chromatography contains a tube called acolumn, into which different chemical substances get into gas stream which rely upon distinctive physical or chemical properties interlinked with column filling. As soon as the substances leave the end point of the column they are validated and examined. The stationary phase which is situated within the column has the capacity to isolate different constituents.

Merits: Sets analysis, typical minutes.

- Efficient, provide high resolution.
- Inexpensive.
- Reliable and relatively simple.

Demerits: These are restricted to the substances which can be easily converted into vapours.

- It is not at all suitable for thermal labile substances.
- They are difficult for huge samples (EL-Maali *et al.,* 2015).

5. Paper chromatography -This type of chromatography is basically used for the alkaloids identification, purity, and validation. For carrying out this process a tissue paper is used and its color is very important for checking out and for comparing it with standards. For the identification of alkaloids, the solution of alkaloids is being transferred onto the tissue paper. This technique is referred to as both analytical methods i.e. qualitative and quantitative. In this chromatography technique the paper which is being used in the process resembles to that of thin layer chromatography but that doesn't make any differences and there is no need of specific coating. In today's time period various modern chromatography techniques are being used and those are based on the basic principles of this method, in which we do consider various forma i.e. splitting and actual behaviour of the compounds and their constituents in the both phases i.e. stationary as well a mobile phase (Cieśla, 2012).

Merits: This technique needs less quantity material.

- This type of chromatography is less costly than others.
- Paper chromatography helps in the identification of various organic an inorganic substance.

Demerits: Paper chromatography cannot handle large quantity of sample.

This type of chromatography cannot separate complex substances (Komsta *et al.*, 2011).

6. Column chromatography- Chromatographic methods are used to purified the characteristics like size, shape, net charge, stationary phase used and binding capacity of proteins (Zweig *et al.,* 1973). Among these methods, this is

the most prominent method for purification of molecules. The column present in this instrument is used for the placement of sample and then mobile phase respectively. There is a fiberglass present inside the instrument where we ensure the flow of the material which was placed inside. The materials at the bottom of the instrument are being collected in a definite time and volume.

Merits: This type of chromatography is used for analysis and its applications.

- This helps in the identification of the constituents present in the mixture.
- It is used for many types of mixture for separation.

Demerits: Time consuming process.

More amount of mobile phase required (Gong *et al.*, 2000).

7. Ion exchange chromatography- This type of chromatographic method involves electronic interlinking between proteins which are charged and supporting material which is solid in nature. The basic process of separation involving the charge of ions i.e. if the material has an opposite charge as compared to protein then the probability of separating them is high. If we alters pH, concentration of salts and ions of the solution then we can separate proteins from the column (Li *et al.*, 2003). Positively charged ion material is known as the anion exchange material which absorb negatively charged proteins. While the negative charged ion-exchange material is known as the cation exchange material which absorb positively charged proteins.

Merits: This type of chromatography helps in increasing the life of resins.

- This type of instrumentation is cheaper to maintain.

Demerits: Nature and properties of ion exchange resins.

Nature of exchanging ions (Shulammithi *et al.*, 2016).

8. Gel permeation chromatography- In this method, dextran containing material is used to separate macromolecules having different weights. This type of chromatography technique is usually utilised for the purpose of identifying various parameters of proteins i.e. their molecular weights and concentrations of salts. In this technique, stable phase constitutes substances with small holes. It constitutes of a part called as column through which the substances pass simultaneously with perpetual flow (Liu *et al.*, 1993).The substances which are of size which are larger than the holes cannot move through the gel and they are being restricted their itself. The substances which are larger can easily move from the pores whereas the substances whose size is smaller than the holes they diffuse and moves out of the column. The solution which contains different dimensions are passed continuously with a constant flow rate through the column.

Merits: Short analysis time.

- Well defines separation.
- There is no sample loss.
- Small amount of mobile phase required.

Demerits: In this type of chromatography filtration must be done before using it (Williams, 1970; Tung, 1971).

Conclusion

There are various phytochemicals obtained from the plants which have medicinal properties. These bioactive compounds from a plant can be extracted using different extraction techniques. There are different methods of extraction depending upon the plant and nature of phytoconstituents which are to be extracted. After isolation, extraction the extract contains a complex mixture. Therefore, with the help of different chromatographic techniques, we get the sample in its purest form. The phytoconstituents obtained thus serve an important role in the prevention and treatment of various diseases.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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