A HAND BOOK ON: SEMI MICRO TECHNIQUE FOR EXTRACTION OF ALKALOIDS

By

Dr. G. Madhumitha & Ms. J. Fowsiya

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PREFACE

A Hand Book on: Semi Micro Technique for Extraction of Alkaloids published by ISCA provides information on the alkaloids, its classification and the various methods such as conventional and non-conventional methods involved in the extraction of alkaloids from different sources. This hand book serves as a dictionary on extraction of Alkaloids for under graduate and post graduate chemistry students. First, considerably greater emphasis has been placed on extraction and identification of compounds from the natural source by simple chemical tests. Secondly, whilst retaining undiminished the full and clear directions provided for students who are studying the Phytochemistry/ Pharmacognosy/ Natural Product Chemistry. We have extended the scope of the work so that it covers most of the needs of students working for an Honors/Special degree. These experiments form the central building in pillar in Phytochemical approach around which most of the recent research developments revolve. We have maintained the standard which was self-imposed when this book was written, namely, that all the experiments in the handbook had been critically examined.

DR. G. MADHUMITHA

&

Ms. J. FOWSIYA

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1. Introduction

Phytochemistry deals with chemical constituents of plants, phytochemicals structure, and their biosynthesis and biological activities. Phytochemicals referred as biological active components of plants. The plant constituents were classified into primary and secondary metabolites. The primary metabolites are sugar, amino acids, nucleic acid, etc. Whereas secondary were alkaloids, anthocyanin, flavonoids, terpenoids, etc., [1]. The quantities of these metabolites vary from species to species [2]. The Alkaloids were the most important group of secondary metabolites found in living organism. Alkaloids never occur alone, they were a mixture of few major and few minor alkaloids. It has different types of structure, properties and pharmacological activities. Most of the alkaloids contain nitrogen in negative oxidation state and also in addition to carbon, hydrogen it contains sulphur, oxygen and phosphorus.

In 1803 semi-pure alkaloid was isolated by Derosne and in 1805 Serturner isolated and characterized the opium alkaloids from *Papaver somniferum*. The first synthesized alkaloid was coniine from *Conium maculatum* in 1886. Strychnine, Emetine, Brucine, Piperine, Caffeine, Quinine, Cinchonine and Colchicine alkaloids are the cornerstone of all that has occurred in alkaloids chemistry to the present day [3]. Most of the alkaloids were derived from amine by decarboxylation of amino acids.

The Alkaloids are biologically active compounds used for anesthetics, sedatives, stimulants, Tranquilizers. Today, several alkaloids are still useful such as caffeine used for the mental alertness, Cocaine used as anesthetic and Morphine used as the painkiller [4].

The present study is on the extraction of known and unknown alkaloids from varies plant species with new techniques of modern science. This handbook provides an easy and brief explanation for the different extraction methods and qualitative tests for alkaloids.

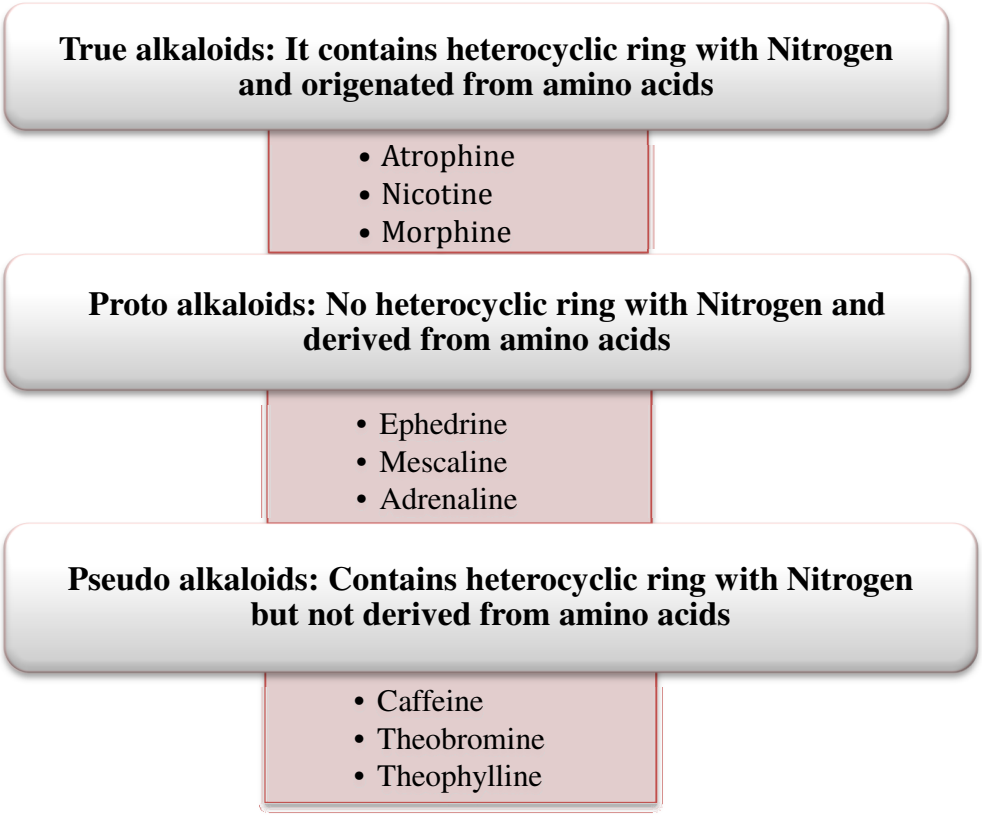
2. General characteristics of alkaloids

Most of the alkaloids are bitter taste, weak base, colorless, poorly soluble in water and readily soluble in organic solvent such as diethyl ether, chloroform, etc., a few alkaloids are colored such as Berberine is yellow color and salt of Sanguinarine is copper Red color. The

Alkaloids will be decomposed by heat except strychnine and caffeine. Mostly the physical form of alkaloids is crystalline solids and few are amorphous solids [8].

3. Classification of alkaloids

When compared with other class of naturally occuring conpounds, there is no uniform structure classification for alkaloids. Present days alkaloids classified based on the carbon skeleton that present in the alkaloids [9].



Scheme 1. Types of Alkaloids

Apart from the above classification, the following methods can be employed in the classification of alkaloids, they are as follows.

Biosynthetic classification	Chemical classification	Pharmacological classification	Taxonomic classification
• Indole • Piperidine • Pyrrolidine • Phenylethylamine • Imidazole	• Tropane • Quinoline • Purine • Diterpene • Steroidal	• Morphine • Quinine • Lobeline • Aconitine • Ergonovine	• Cannabinaceous • Rubiaceous • Solanaceous

Scheme 2. Classification of alkaloids

In general, alkaloids classified into two division, i) Heterocyclic ii) Non-Heterocyclic alkaloids

The following are few examples for Heterocyclic and Non-heterocyclic alkaloids.

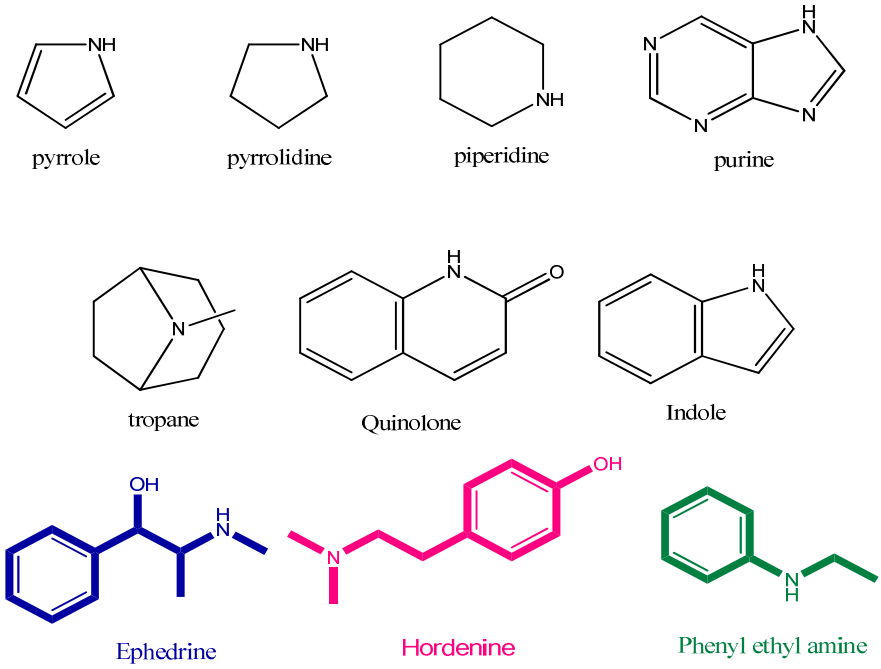
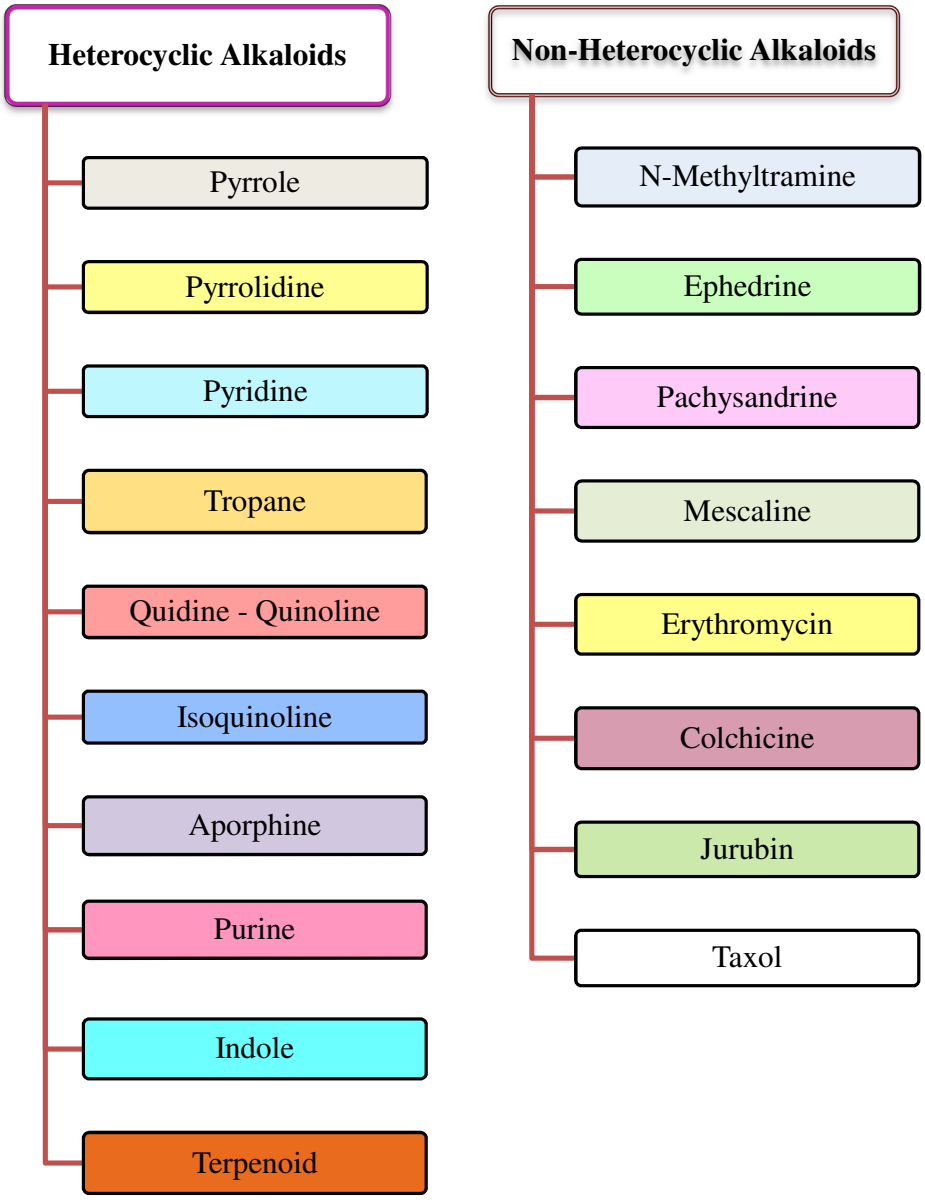


Fig 1. Types of Alkaloids based on their Structure



Scheme 3: A few examples for Heterocyclic and Non-Heterocyclic alkaloids

4. Extraction of Alkaloids

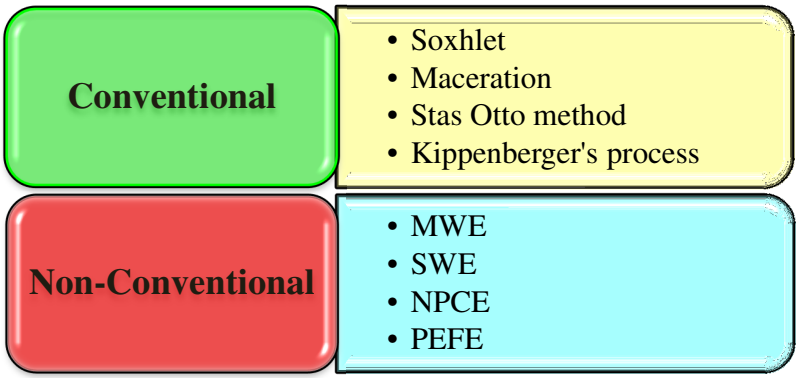
The extraction is defined as the analytical tool for the separation of biologically active components of plants or animals from inert or inactive component by using suitable solvent in Standard extraction procedure. Maceration, percolation, digestion, soxhlet extraction, microwave-assisted extraction (MWE), sonication (USE), super fluid extraction (SFE) is general techniques for the extraction of alkaloids [10]. The extract composition is depending on, extraction

method, temperature, solvent nature, concentration of solvent, polarity, solubility and extraction time. These traditional methods have some limitation including, long time extraction, solvent consumption, less efficiency etc., but in recent year new extraction techniques have been developed with more efficiency, less consumption of solvent and easy to isolation[11]. The following extraction methods were outstanding development in the area of extraction of different alkaloids, spectroscopic techniques, to determine activity of alkaloids and structural elucidation.

4.1. Extraction methods of alkaloids

The following methods were used for the extraction of alkaloids from various natural sources.

1. Soxhlet extraction.
2. Stas-otto extraction
3. Kippenberger's process
4. Manke's process
5. Microwave assisted extraction (MAE)
6. Ultrasonic assisted extraction (UAE)
7. Subcritical water extraction (SWE)
8. Maceration
9. Negative pressure cavitation extraction (NPCE)
10. Accelerated solvent extraction (ASE)
11. Heat reflux extraction
12. Pulse elecric field extraction (PEFE)
13. Merck process
14. Thiboumery and Mohr process
15. Robertson Gregory process



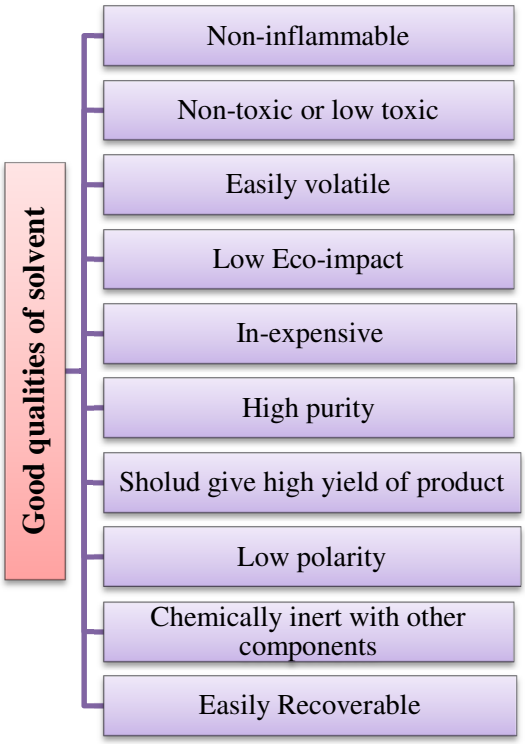
Scheme 4: Conventional, Non-conventional method

4.2. Sample preparation

The fresh plant material was used for the extraction and the source has been kept in alcohol for few minutes. But most commonly dried material of the plant species were used for the extraction. The drying method should be carried out at controlled condition without using high temperature in air drier. This dried plant can be stored in a container for long time. The plant material is reduced to a coarse powder using grinders and sieves to facilitate maximum effective contact of the solvent with alkaloids and tissues.

4.3. Choice of solvent

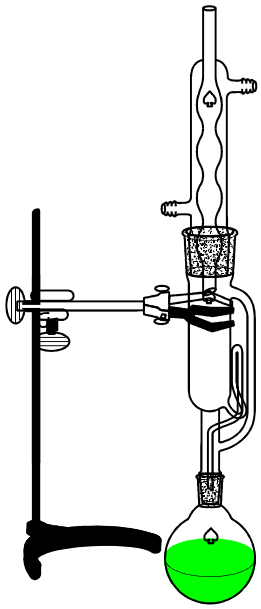
The solvent act an important role in the extraction process and its choice depends on the plant material. Commonly alcohol, ethyl acetate, chloroform and water were used as solvent to obtain high yield. The alcohol used for the pretreatment is to remove chlorophyll and impurities. The solvent should possess the following property such as low toxic, ease in handling and storage, chemically inert. A good solvent must have the following properties,



Scheme 5: Good qualities of solvent

4.4. Soxhlet extraction

The main advantage of the Soxhlet extraction is, it is continuous extraction process, less extraction time and sample gets continuous contact with solvent, no filtration required and low cost. There is some drawback of this method when compared with other techniques. The drawbacks are, large amount of extractant wasted, sample should be boiled over boiling point of solvent for long time which cause decomposition of thermo-labile component and more solvent consuming method [12]



- The powdered plant material taken in the fat free cellulose extraction thimble and covered with cotton.
- A suitable solvent is added to the chamber.
- Soxhlet chamber, condenser and collecting flask assembled in correct manner and heat the setup under reflux [13].

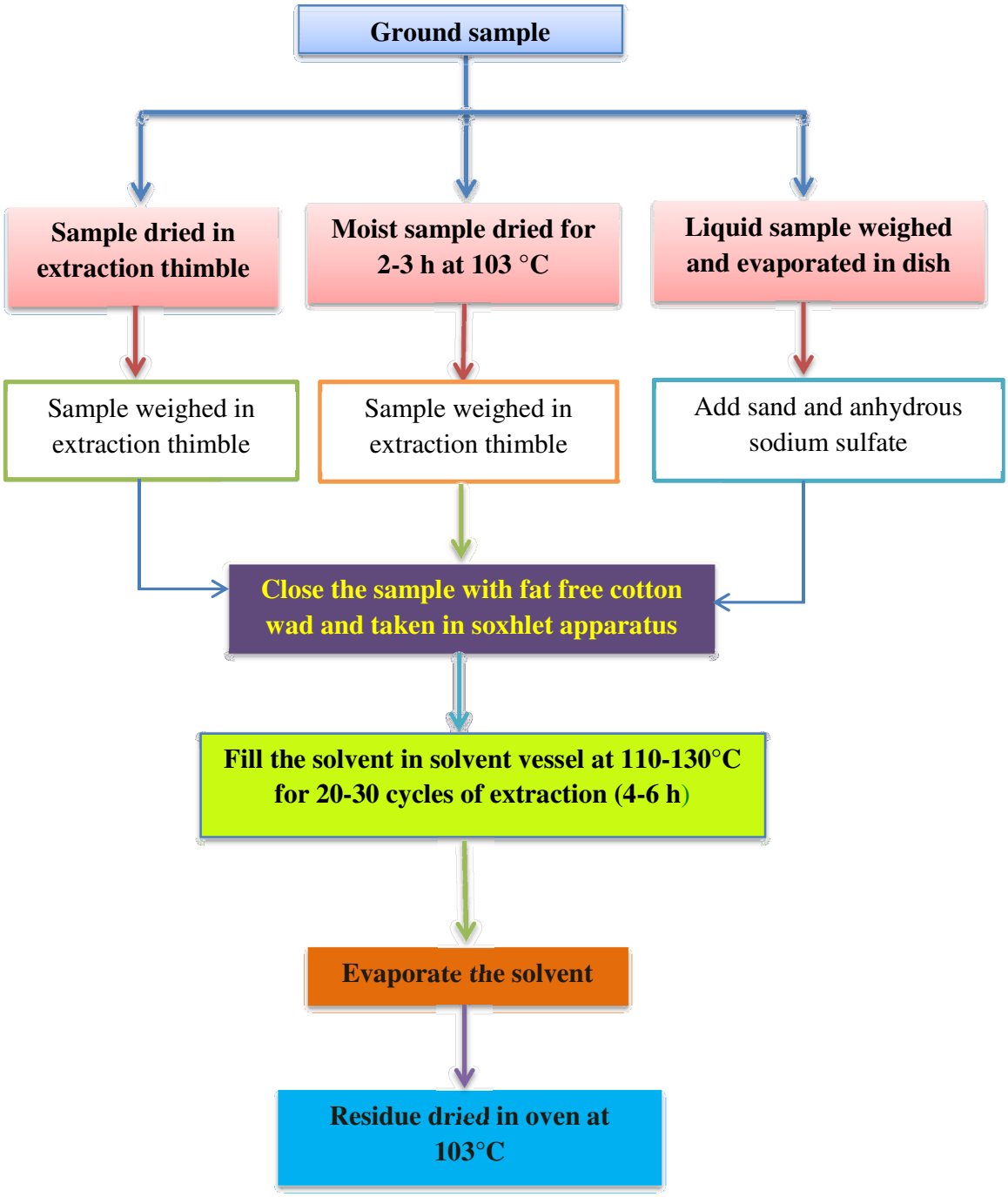


Fig. 2: Soxhlet Extraction

4.5. Stas otto process (Method-1)

Method 1: The solid ingredients were moistened with appropriate amount of solvent and allowed to stand for 4 h in closed container and top of the percolator apparatus is closed. The mixture is allowed to stand for 24 h. The outlet of the percolator then is opened and the liquid contained therein were allowed to drip slowly. Later sufficient solvent is added and the mixed liquid is clarified by filtration or by standing followed by decanting [14].

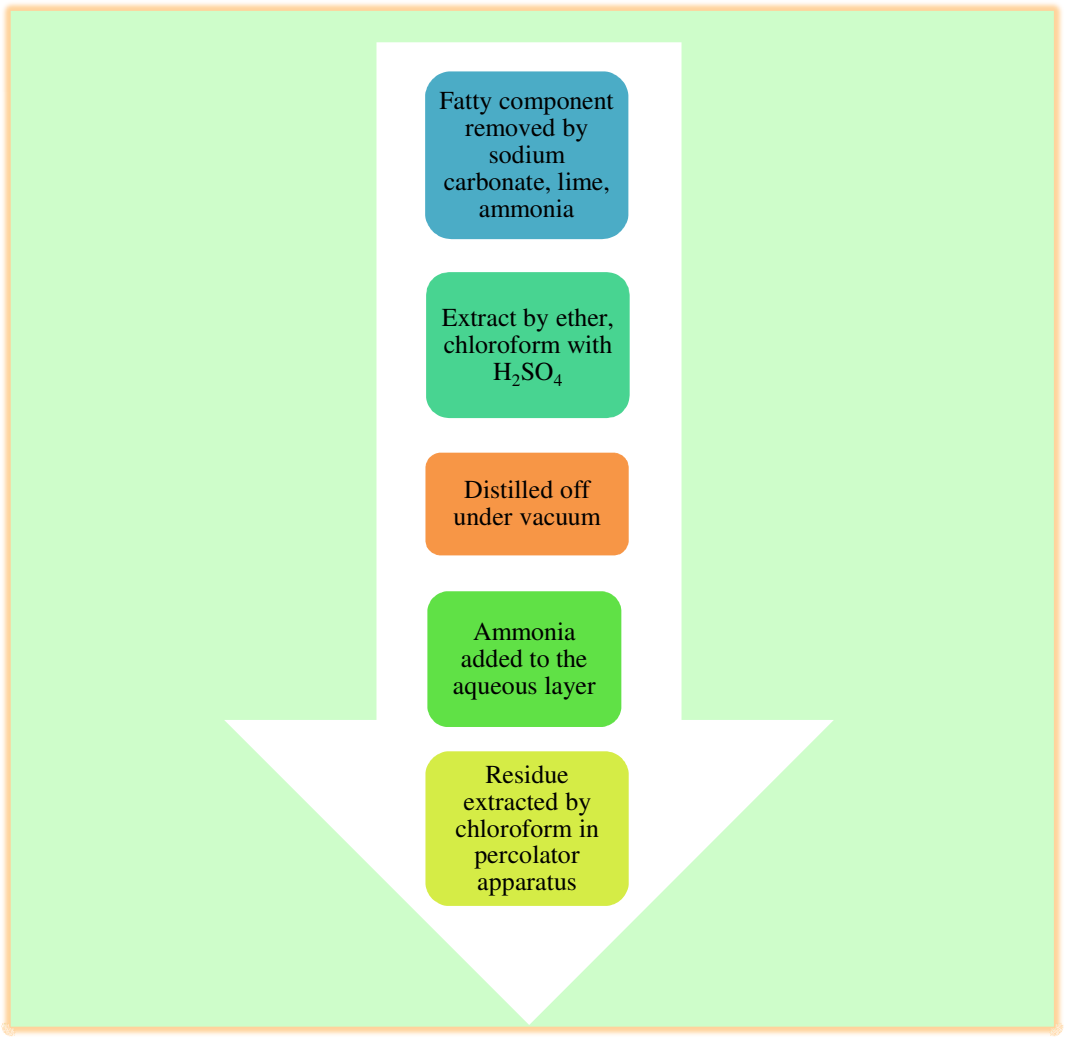


Fig 3: Stas Otto process (method-1)

4.6. Stas otto process (Method-2)

The Powdered plant material was acidified with tartaric acid and then extracted with Ethanol (90-95%). The alcohol portion was then distilled off under vacuum condition. Later Petroleum ether was added to the aqueous residue to remove fatty components. Again the aqueous portion was filtered and evaporated in Rotary evaporator [15].

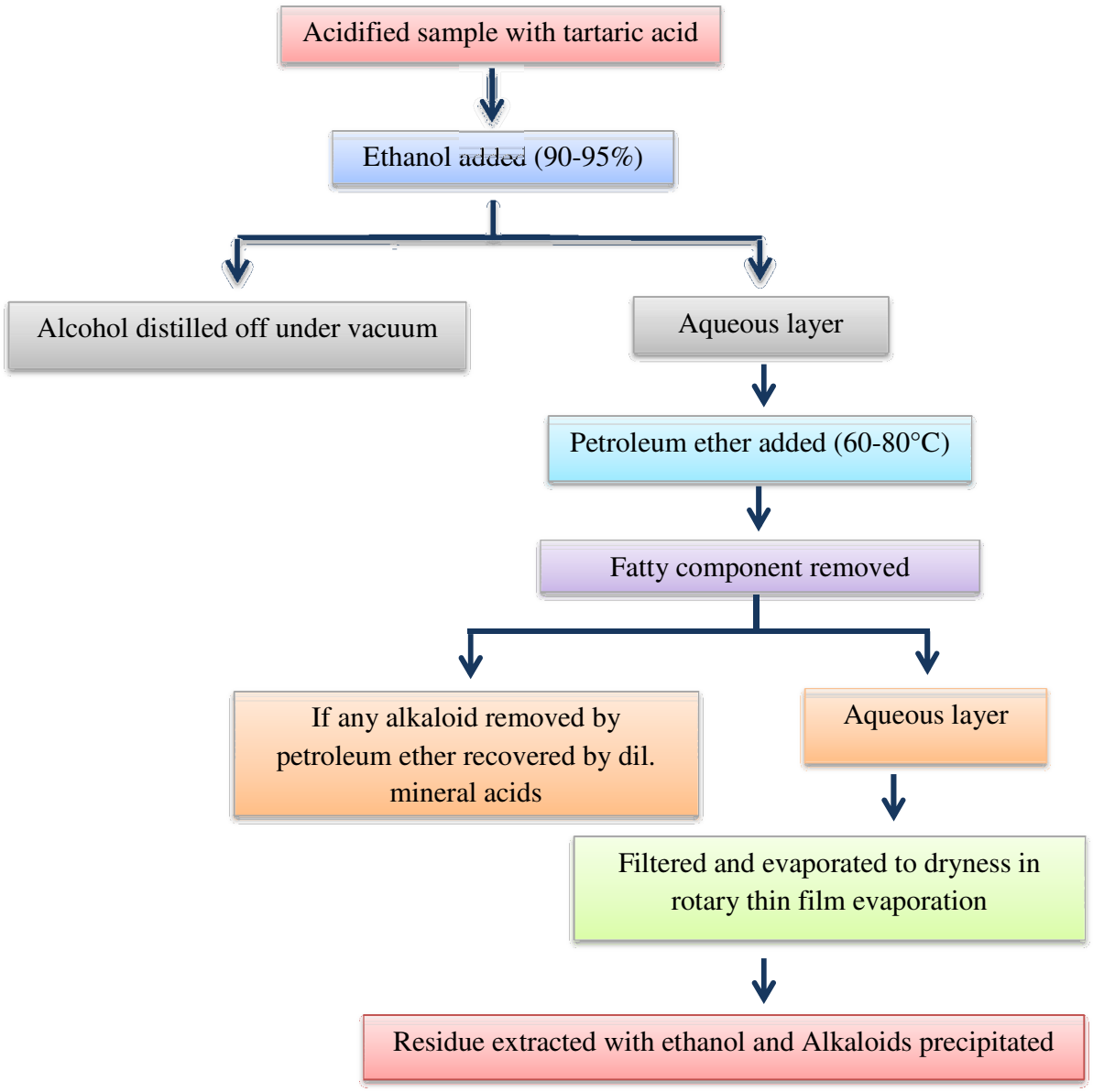


Fig 4: Stas Otto process (Method-2)

4.7. Kippenberger's process

In the Kippenberger's process, the powdered sample was digested with tannin in glycerol at 40°C for 48hr. The residue was again heated, then cooled and filtered. To the filtrate the petroleum ether was added to remove all the fatty components. Then the petroleum ether was removed either on electric water bath or exposure to IR lamp. The fat free part was then acidified and shaken well with the chloroform to remove the narcotine, codeine alkaloids. And CO_2 was passed to remove morphine, narceine. Alkali hydroxide converts into carbonate and finally subjected to alcohol and chloroform. Third alkaloids are removed by equal volume of ether and chloroform [15].

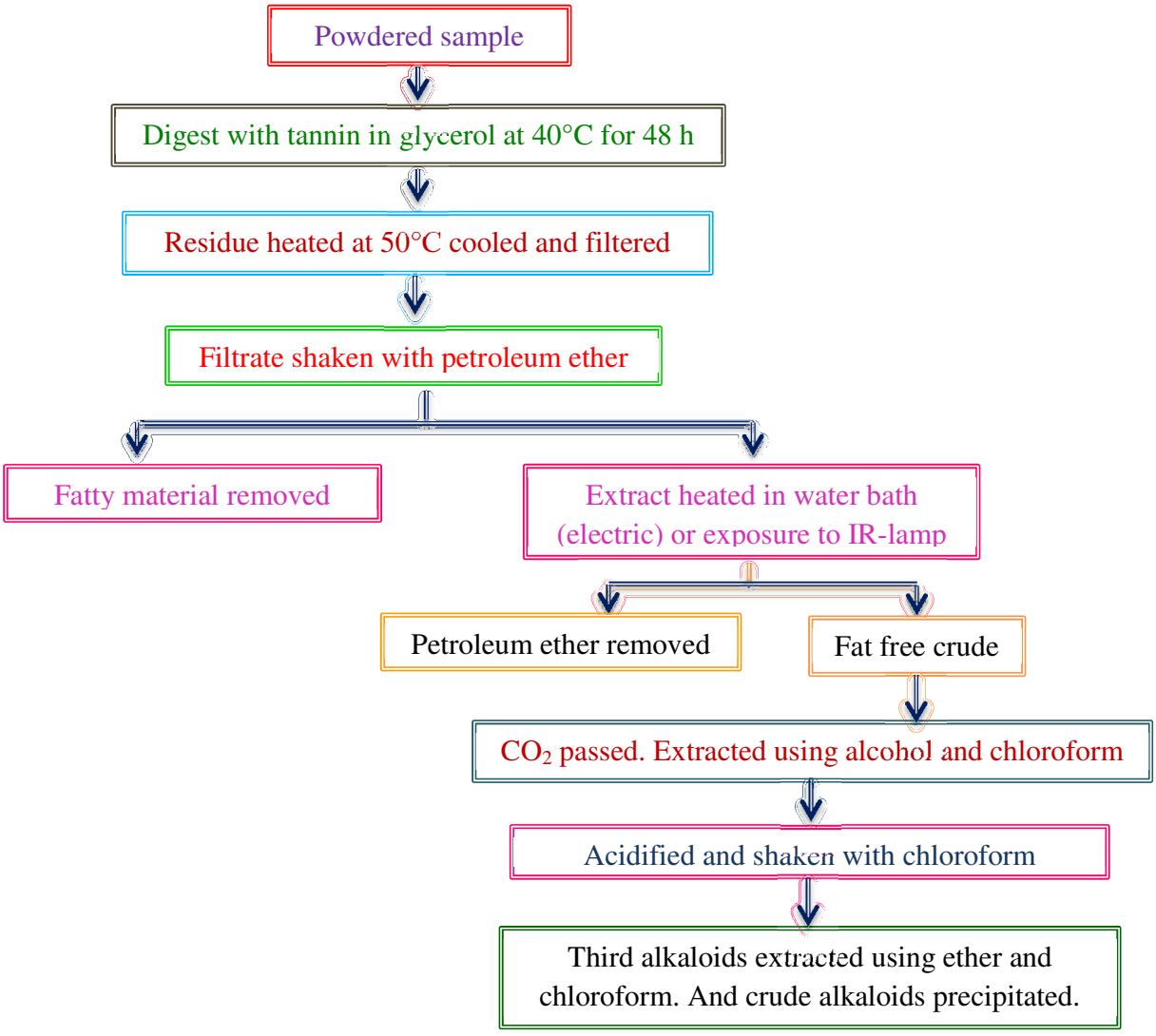
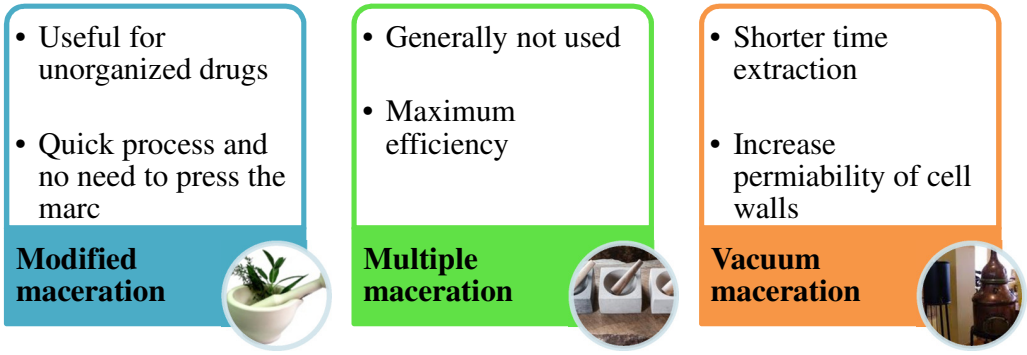


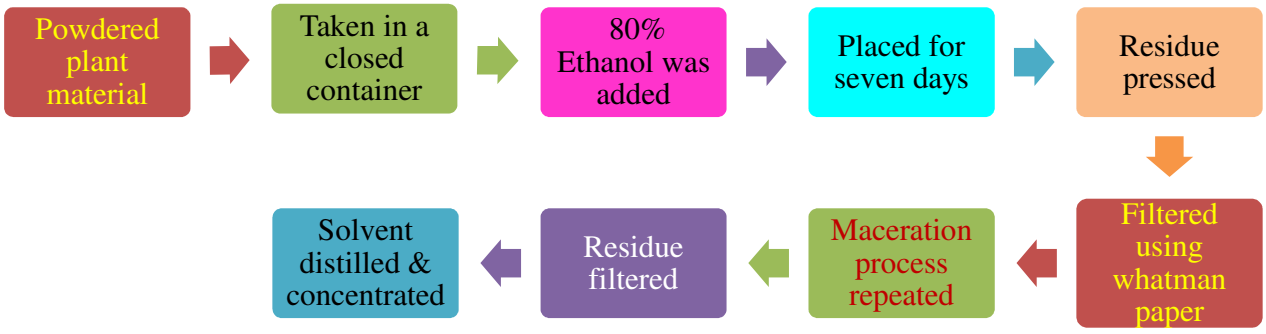
Fig 5: Kippenberger's process

4.8. Maceration

The coarsely powdered plant material was soaked in suitable solvent and kept in closed container for a defined period with agitation to remove all soluble components dissolved in it. After few hours, the marc was then filtered and the residues were filtered using Whatman filter paper. On repeated maceration, the yield extracted will be very high effectively. Maceration extraction is best method for the thermo-labile alkaloids· The maceration method is time and solvent consuming [17]. In recent years, there is new emergence of maceration methodology where, unorganized drugs such as resin, gum, etc., were carried out [17]. The different methods of maceration were listed below.,



Scheme 6: Types of maceration



Scheme 7: Maceration extraction

4.9. Manske's process

The samples were defatted with petroleum ether and alcohol was added to the residue. The alcoholic extract was dissolved in water, the pH were adjusted to 2 and then filtered. To the filtrate ether and chloroform was added, the non-alkaloidal impurities were removed and the organic layer was evaporated and crude alkaloids were collected [18].

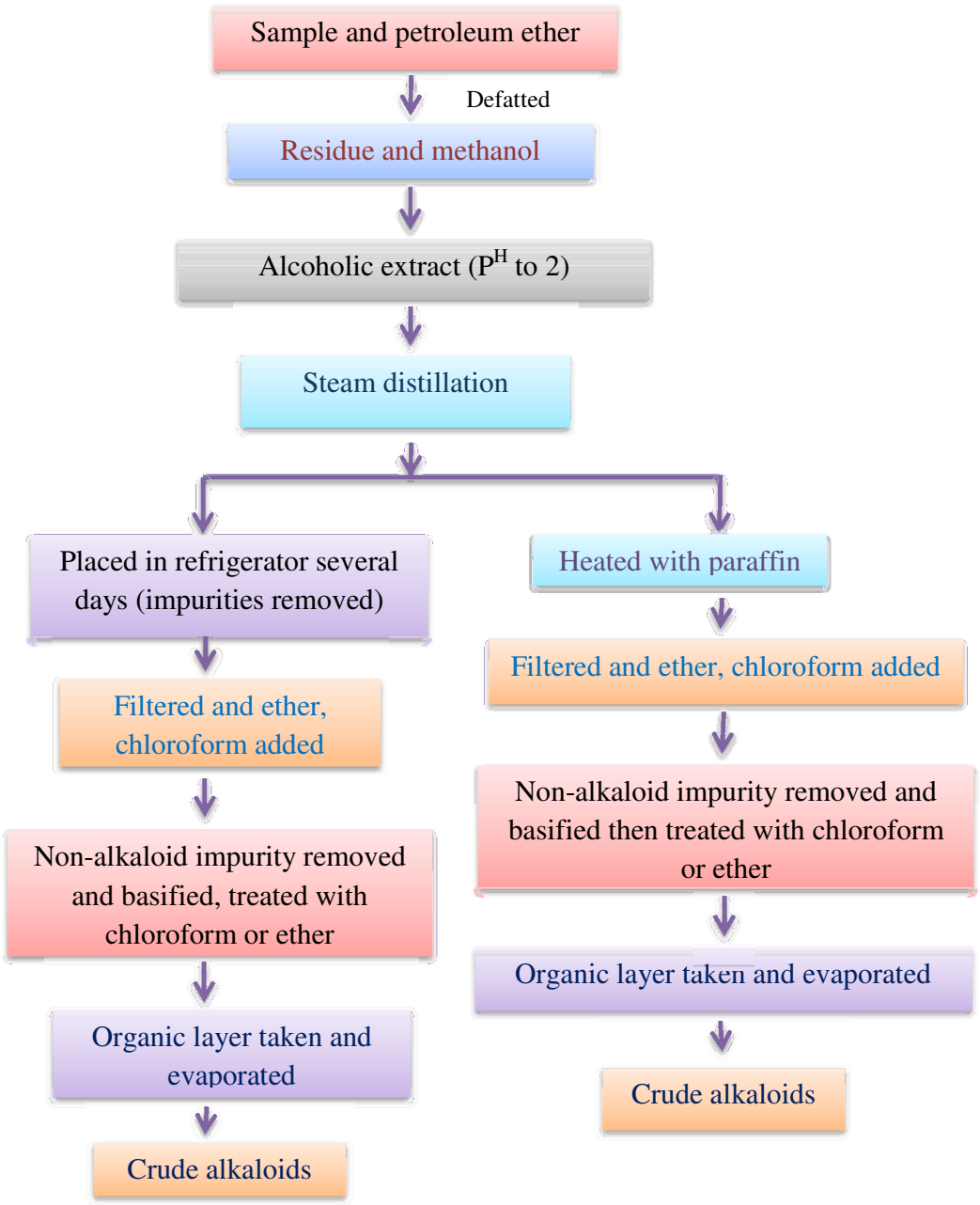


Fig 6: Manske's process

4.10. Negative pressure cavitation

About 25 g of the powdered sample was transferred into the extraction chamber with different concentration of extracting solvent. A vacuum pump was connected to the extraction pot through a condenser to generate negative pressure. The pressure was monitored by adjusting the airflow rate this was supplied from the bottom of the device. After each extraction, the extraction solutions were combined and dried on a rotary evaporator at 45°C. The HPLC-grade methanol was added in a constant volume to prepare the samples for RP-HPLC analysis [19]. This method offers many advantages namely, low cost, reduced noise, low energy, short time extraction, high yield and Eco-friendly.

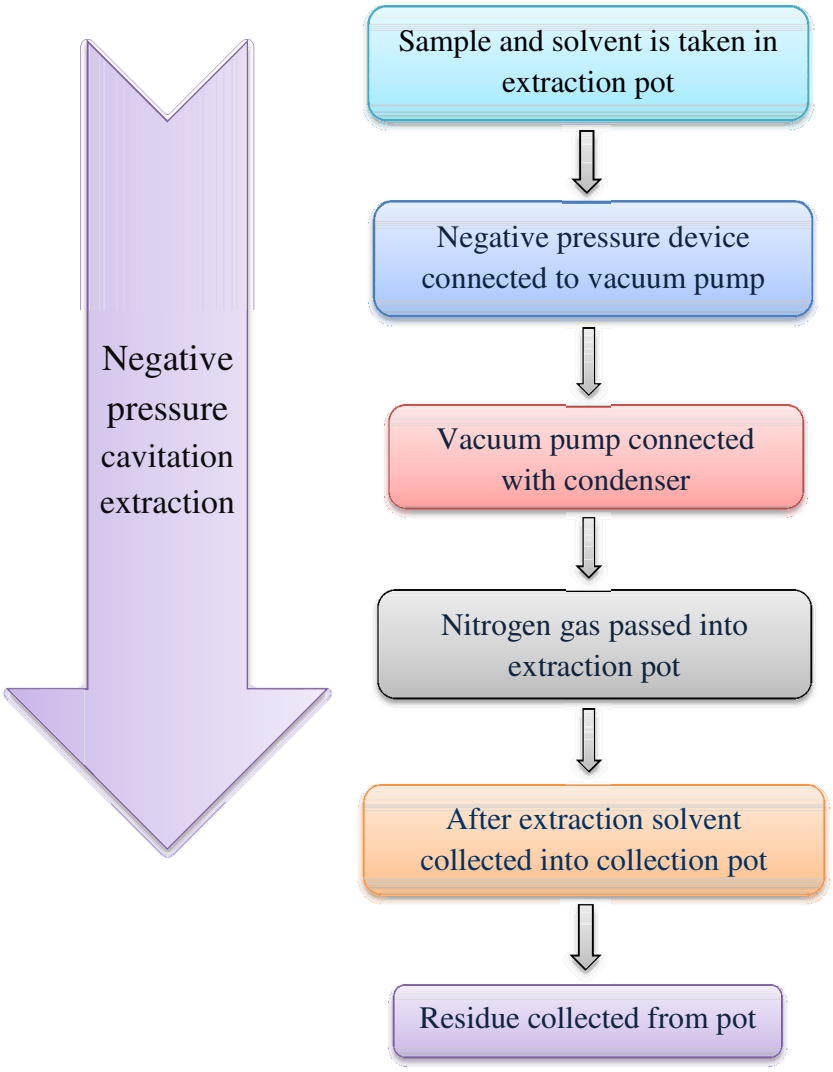


Fig 7: Negative pressure cavitation extraction

4.11. Microwave-assisted extraction (MWE)

The electromagnetic radiation consists of electric and magnetic field perpendicular to each other. The microwave generated in the frequency of 300MHz to 300GHz. This microwave extraction based on the two mechanisms namely, ionic conduction and dipole rotation. The migration of ions under electric field is called as ionic conduction. When the solution is resistance to the migration of ions, then friction is generated and the solution is heated. Therefore, dipole of the molecules changes under electric field and is known as dipole rotation. The heating occurs only at 2450 MHz. microwave interacts with polar solvent and heating occur due to the influence of above two phenomena [20]-[22].

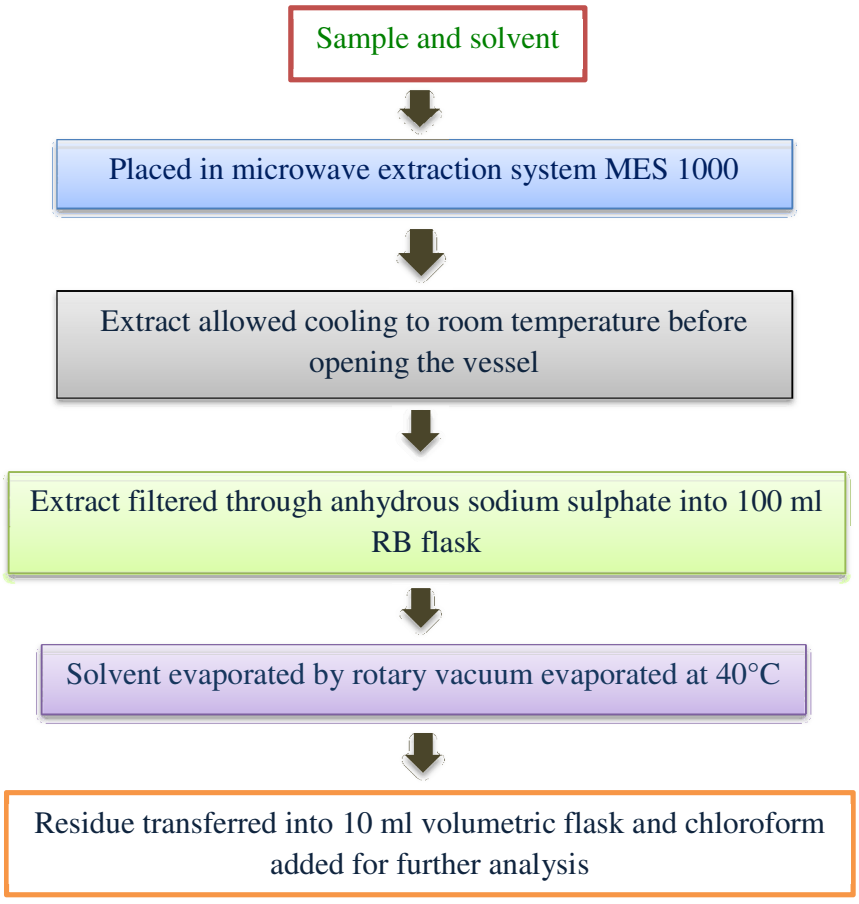


Fig 8: Microwave assisted extraction

4.12. Accelerated solvent extraction (ASE) or pressurized solvent extraction (PSE)

The accelerated solvent extraction method is a new technique which holds, less solvent requirement, single stage extraction, shorter time, and the faster kinetics when compared with other methods. The main advantage of this method is the high temperature and pressure improves metabolite solubilization, decrease in liquid viscosity and accelerate the extraction kinetics which leads to the high yield [23].

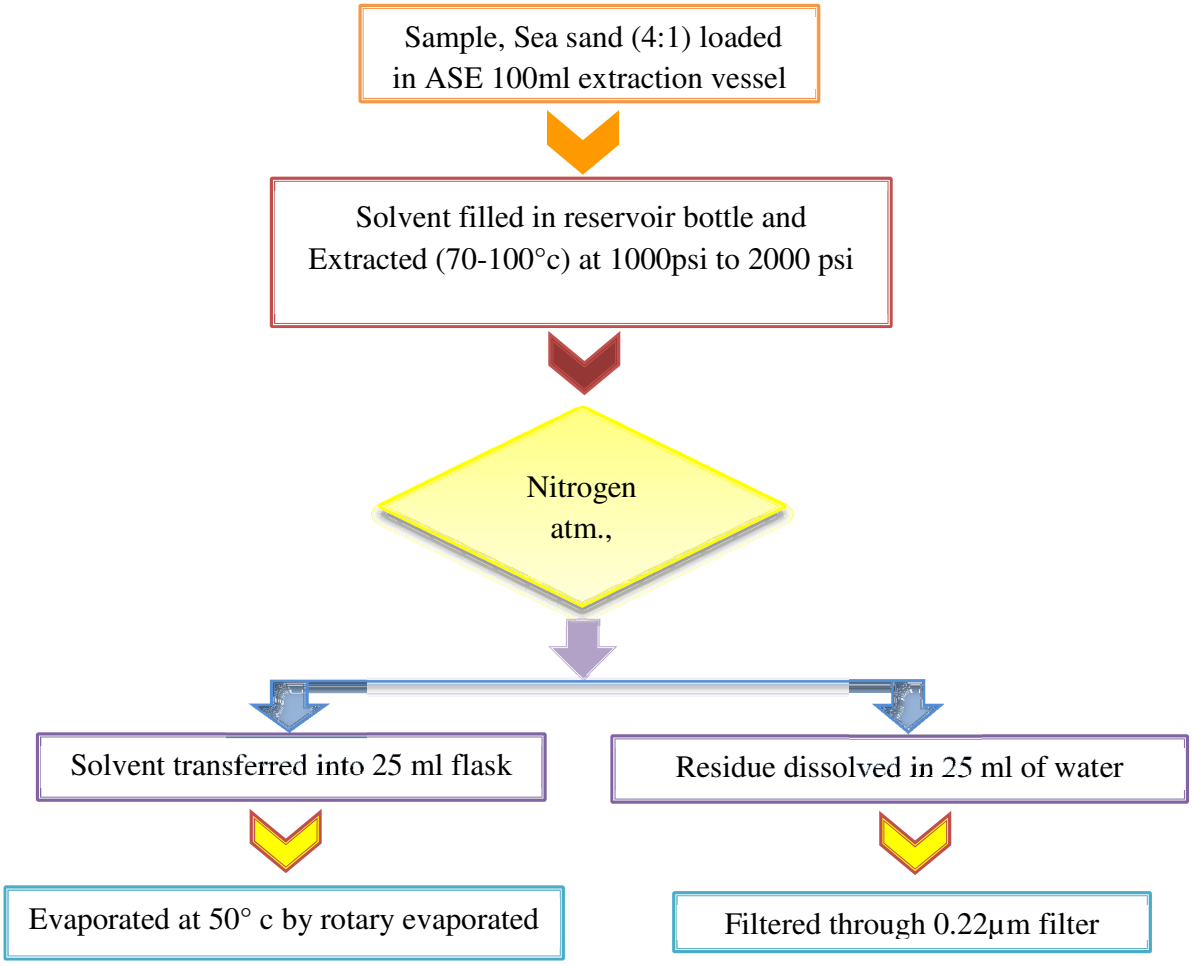


Fig 9: Accelerated solvent extraction

4.13. Subcritical water extraction (SWE)

The Extraction of phytochemicals using hot water (100 to 250°C) under pressure is known as subcritical water extraction. This method works on the bases of dielectric constant of water with temperature. At room temperature dielectric constant of water nearly is close to 80. When water heated up to 250°C dielectric constant becomes 27. Here Reservoir connected to pump to introduce solvent into the system [24].

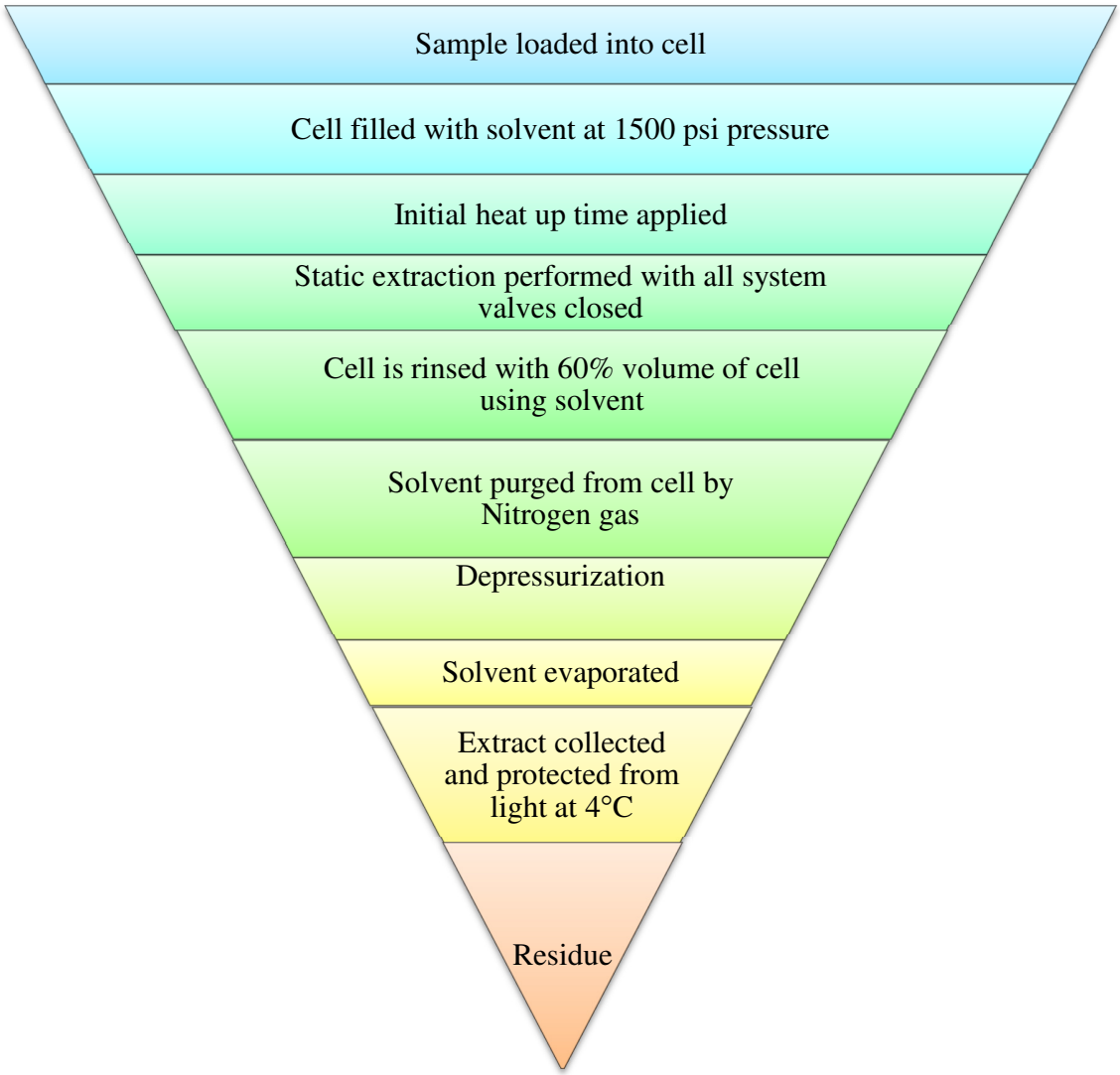


Fig 10: Subcritical water extraction

4.14. Ultrasonic assisted extraction (sonication)

The Sample was transferred into glass container in contact with 50ml of solvent and closed with aluminum foil then placed in ultrasonic bath. The Ultrasonic extraction works from 20 kHz which induce a mechanical stress to produce cavitation on plant material. The High yield obtained in an increase of solubilization of metabolite by breaking of cellular. The extraction was repeated for every 30min and extract was filtered with anhydrous sodium sulphate and the solvent was evaporated in rotary evaporated at 45°C [25].

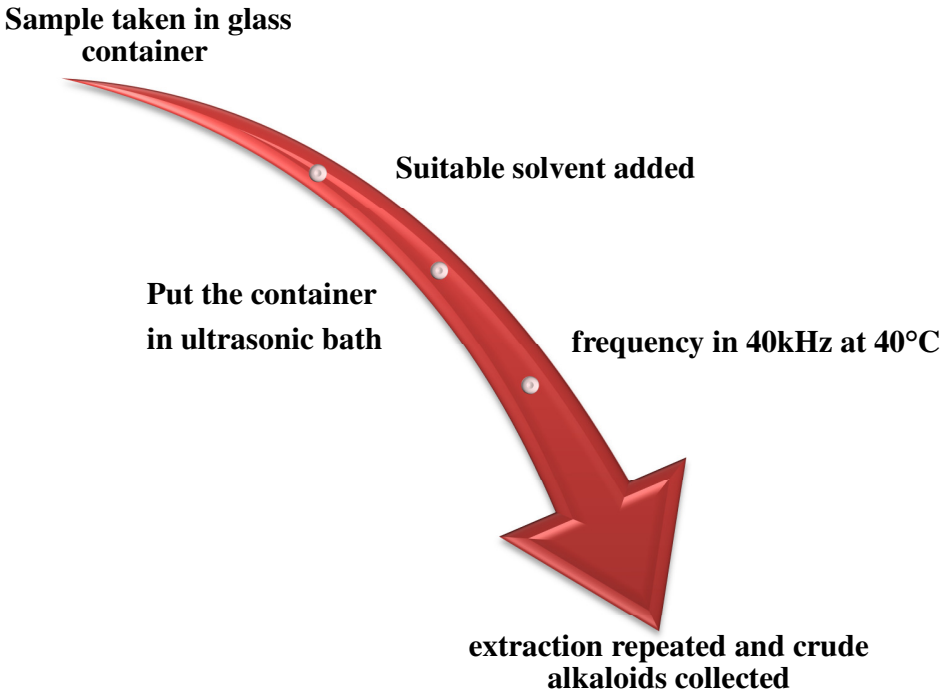


Fig 11: Ultrasonic assisted extraction

4.15. Pulse electric field extraction (PEFE)

The principle of Pulse Electric Field Extraction is to destroy the cell membrane structure to increase the kinetics of the extraction. During the suspension of a living cell in an electric field, the electric potential passes through the membrane of that cell. Based on the dipole nature of membrane molecules, the electric potential separates the molecules according to their charge in the cell membrane. The PEF can increase the mass transfer during the extraction by destroying the membrane structure of plant materials for enhancing extraction and decreasing extraction time. The dried sample was mixed with solvent and soaked for 24 h. The mixture was pumped through the PEF system with a flow velocity of 120 L h-1. After being processed for the desired time, the high voltage pulsing was turned off, the mixture was pre-filtered through a piece of tulle to remove solid particulates, and then through a 0.45-µm hydrophilic polypropylene membrane [26].

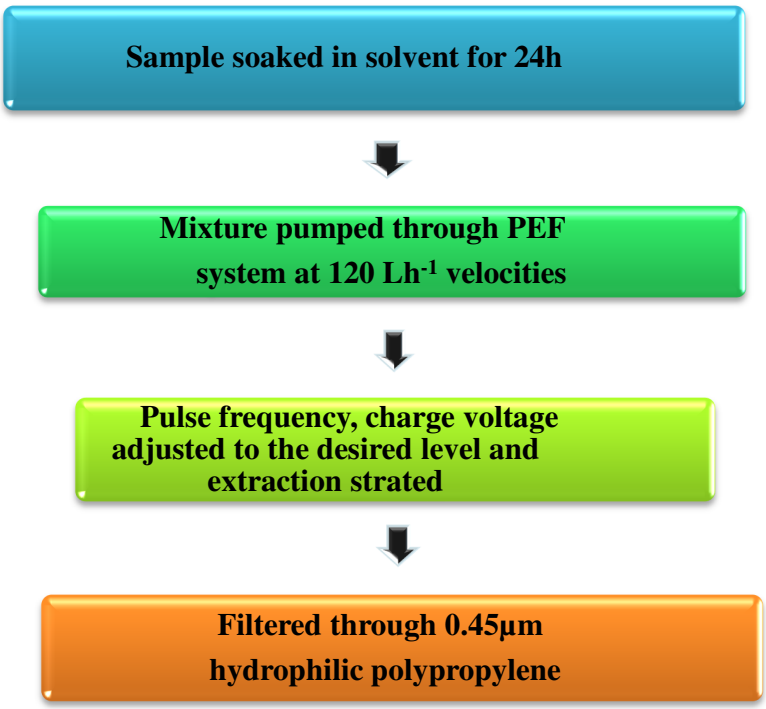


Fig 12: Pulse electric field extraction

4.16. Merck process

In the Merck process, the sample was mixed with cold water and soaked for 24h. The liquid obtained is concentrated and precipitated hot with sodium carbonate. Ammonia is then evaporated on heating the mixture and it should be alkaline to phenolphthalein. After 24h the mixture is filtered, dissolved in alcohol at 85 °C. The excess alcohol is then evaporated and residue mixed with acetic acid for neutralization. This acetic solution is then decolorized with charcoal, filtered and precipitated with ammonia [27].

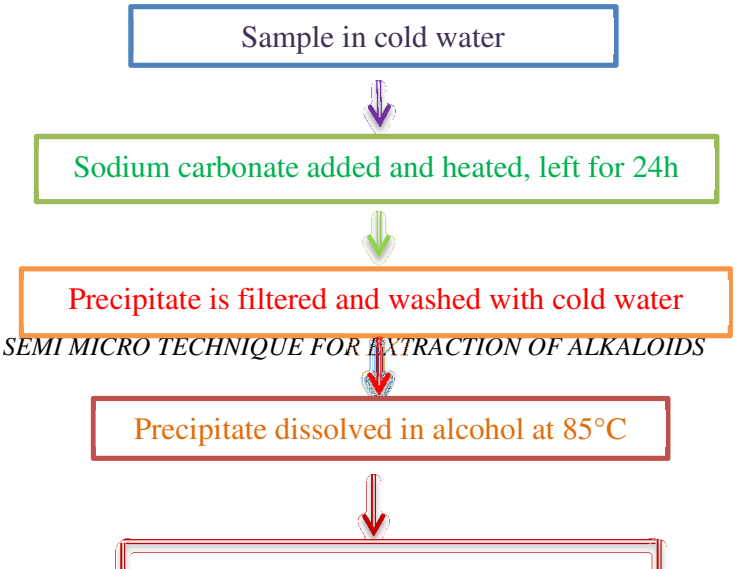


Fig 13: Merck process

4.17. Thiboumery and Mohr process

The sample material was treated with hot water with triplicate. The liquid is filtered off and the residues were again treated with three times its weight of water. The solutions evaporated to half their volume and poured into boiling milk of lime. The precipitate were filtered off and re-treated with three parts of water to one part of opium. It is then filtered off again and the solution was heated to boiling, and the alkaloids were precipitated by the addition of ammonium

chloride. After cooling, it is filtered and the precipitate was washed and then purified by solution in hydrochloric acid [28].

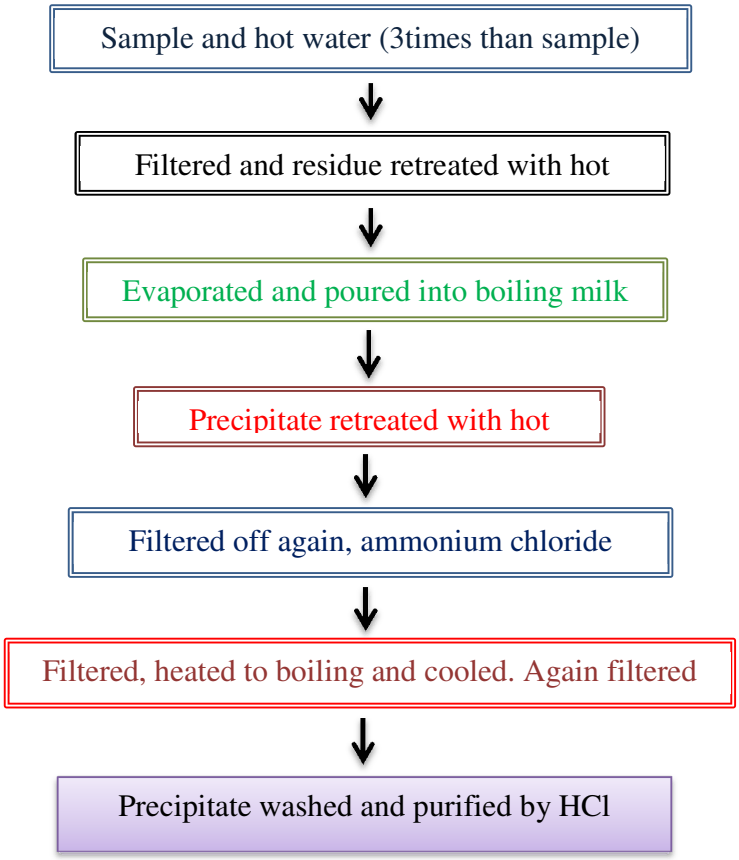


Fig 14: Thiboumery and Mohr process

4.18. Robertson-Gregory process

The plant materials were completely treated with five to ten times of cold water and the solution was evaporated and the process was repeated with cold water. The solution obtained was evaporated and few crystals of calcium chloride added to the boiling solution which is then diluted with cold water. The solution was then filtered and concentrated further. This deposit is filtered off and the solutions were left in on-standing. After a few days, the

filtrated material it becomes a crystalline in nature. The crystals obtained are drained and then placed in a cloth. The solution were then decolorized each time with animal charcoal and dissolved in water and the alkaloids are precipitated with ammonia [28].

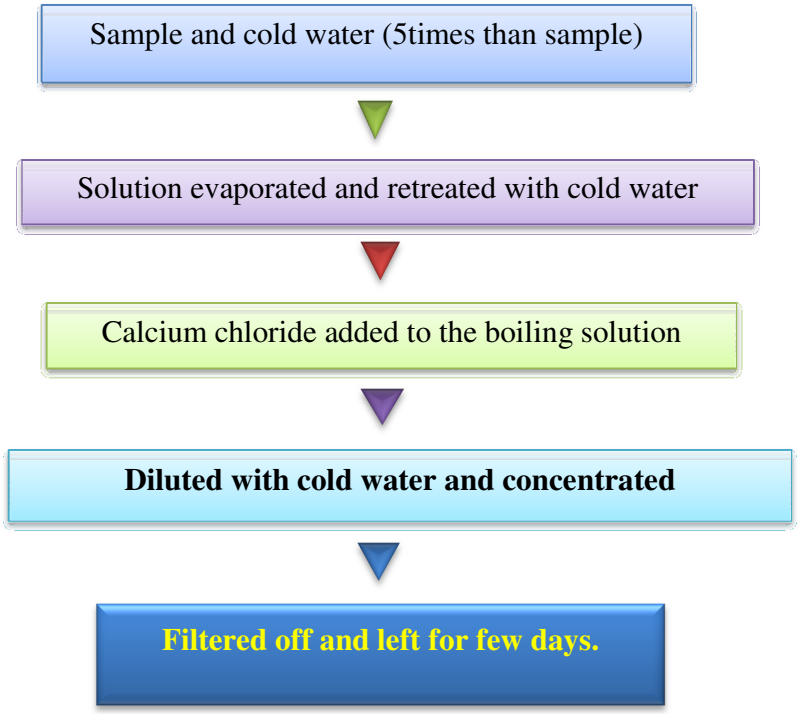


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Fig 15: Robertson-Gregory process

5. Separation of different types of alkaloids [29]-[30]

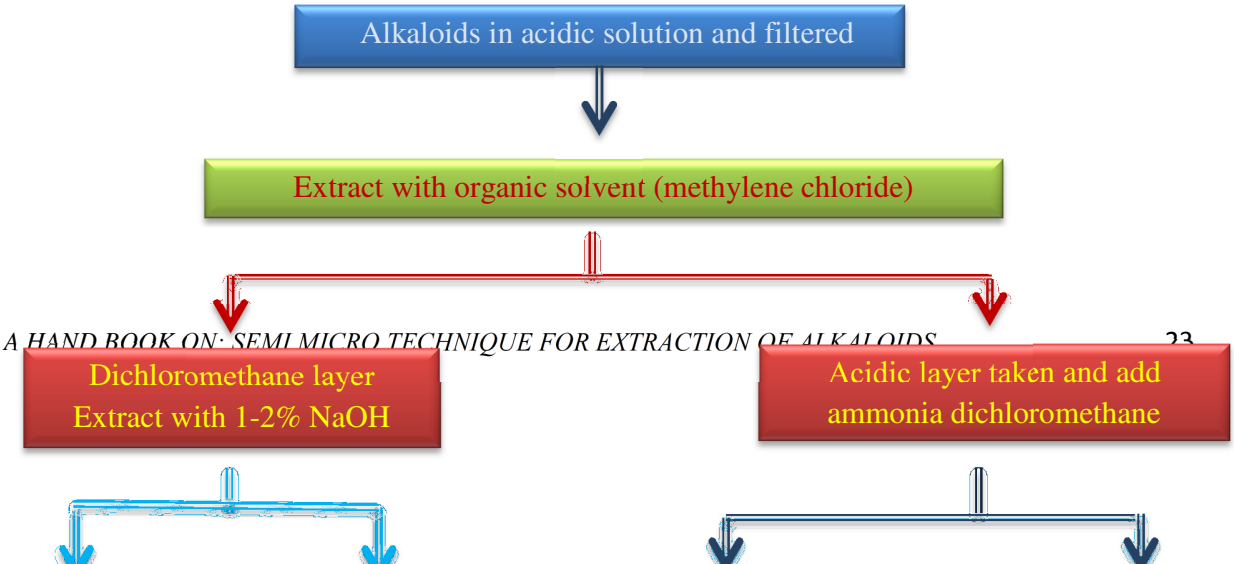

Fig 16: Separation of Alkaloids (A)

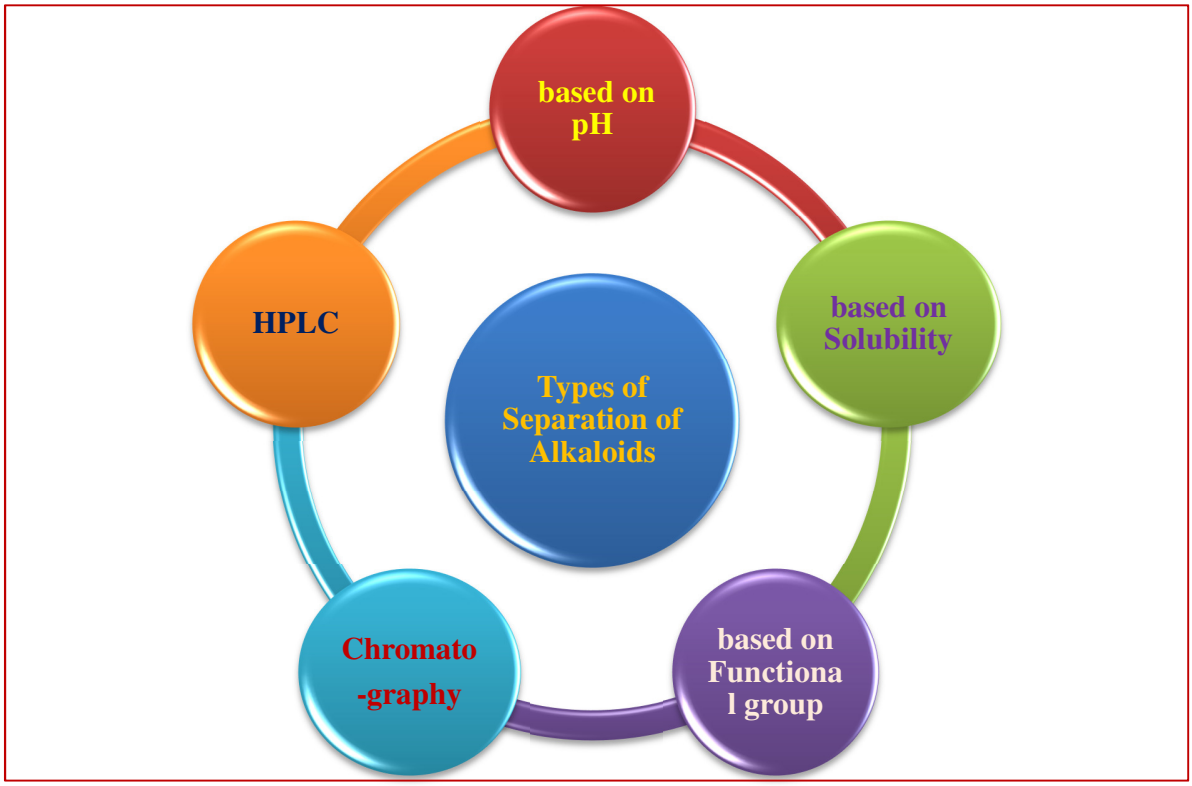

Fig 17: Separation of Alkaloids (B)

6. Qualitative analysis of alkaloids

The qualitative analyses of alkaloids are important study for the quantification of alkoaids in a particular crude extract. The concentrated extract solution was used for the investigation of alkaloids for the lack of satisfactory crystals formation. When alkaloids or alkaloids salts were not dissolved in water, 10% HCl was used to dissolve the crystals. These solutions are taken for the test with different reagent. The crystals formed are visible under microscopic condition. Few drops of test solution and reagent added on glass rod and examine for microscopic condition.

Sometimes the crystals not identified quickly. Hence care should be taken and stir the solution to form crystals. The stirring with glass rod is avoided on the account of abnormal result of crystals. Crystal shows variation upon amount of reagent added. Normally few ml of test solution were needed to the formation of crystals. The following table consists of some chemical test for alkaloids. Different class of alkaloids gives different color with reagents.

Table 1 Chemical test for Alkaloids [31]-[36]

S.no	Experiment name	Chemical required	Inference
1	Mayer's Test	1.36g of Mercuric chloride, 3g of Potassium iodide in 100 ml of Water	Cream color Precipitate
2	Wagner's Test	1.3g of iodine and 2g of potassium Iodide	Brown or Reddish Color precipitate
3	Kraut's Test	8g of Bismuth Nitrate, 20ml of Nitric acid and 27.2g of Potassium Iodide in 100ml of Water	Brown color precipitate
4	Marme's Test	10g of cadmium Iodide boiled in 100ml of Water then 20g of Potassium Iodide added	White color precipitate
5	Scheiblers Test	20g of sodium tungstate, 70g of Disodium phosphate in 100ml of water	White color precipitate
6	Hager's Test	Saturated Picric acid	Yellow color precipitate
7	Sonnenschein reagent test	1% solution of Phospho molybdic acid in Ethanol	Orange or Red color precipitate
8	Bertrand's test	Silicotungstic acid in distilled water	White color precipitate
9	Reineckate salt solution	1g of Ammonium Reineckate, 0.3g of Hydroxyl Amine Hydrochloride and 100ml of Ethanol	Pink color precipitate
10	Froehde reagent Test	5g of Molybdic acid or 5ml of Sodium Molybdate in 5ml of concentrated Sulphuric acid	Blue-Green color precipitate
11	Erdmann's reagent Test	10drops of con. Nitric acid, 100 ml of water is added to 20ml of con. Sulphuric acid.	Cream color precipitate
12	Marquis reagent test	3drops of Formaldehyde (40%) and 3ml of Sulphuric acid.	Orange-Green

13	Mandelin's Reagent Test	1g of Ammonium vanadate, 100ml of con. Sulphuric acid.	Olive black
14	Mecke's reagent Test	1g of Selenious acid mixed with 100ml of con. Sulphuric acid	Red color precipitate
15	Rosenthaler Reagent Test	1g of Potassium Arsenates in 100ml of con. Sulphuric acid	Red color precipitate
16	Folin-ciocalteu's phenol Test	Folin reagent and equal volume of distilled water	White color precipitate
17	Van Urk reagent Test	Para Dimethyl Aminobenzaldehyde in 65% of Sulphuric acid or HCl	Reddish brown color precipitate
18	Dragendorff's reagent test	Potassium Bismuth Iodide	Reddish Brown color precipitate

Test for Aconitine [37]

1. Amorphous precipitate from in the solution of sodium carbonate
2. Slowly crystals form with potassium permanganate.
3. Slowly crystals form with potassium hydroxide

Test for Anhalonine [37]

1. Precipitate formed with Kraut's reagent(1:200)
2. Stirring with 1:50 solution of Gold chloride a large rods are formed
3. Amorphous precipitate formed with picric acid. With more dilute solution we get small solids.
4. Alkaloids with Wagner's reagent form a heavy amorphous precipitate.

Test for Brucine

1. With concentrated solution of potassium chromate rosettes are formed.
2. Small crystals are formed with Platinum chloride.
3. Many of the needles formed with Potassium ferricyanide.
4. Million's reagent gives a rose-red color with alkaloids.

Test for Caffeine

1. Needle shaped cyrstals formed with Mercuric chloride.
2. Very small and amorphous precipitates formed with Silico-tungstic acid.

Test for Coumarins [38]

1. To the alkaloid 1N sodium hydroxide added, test tube covered with filter paper and heated in water bath and examined for UV light for yellow fluorescene.
2. Add few ml of chloroform and 10% sodium hydroxide yellow color formed.

Test for Choline

1. Heavy Amorphous precipitate formed with phosphortungstic acid.
2. A large number of small crystals formed with Barium nitrate.

Test for Cocaine Hydrochloride

1. Purple colored plates are formed with Potassium Permanganate.
2. Yellowish precipitate formed with Chromic acid and a small amount of concentrated Hydrochloric acid.
3. Ferric chloride added to the alkaloids and stirred well. A blade like crystals formed with chisel shaped ends.

Test for Codeine

1. Amorphous precipitate formed with Marme's reagent.
2. Yellow color precipitate obtained by adding Million's reagent

Test for Heroine (Diacetyl Morphine)

1. Rosettes of rods obtained with sodium phosphate.
2. Rosettes are formed with 1:200 solution of potassium cyanide.

Test for Hyoscyamine

1. Vitali-morin's test: Alkaloid treated with fuming Nitric acid and evaporated to dryness. To the residue add acetone

Test for Morphine

1. Marme's reagent: this is the best test for morphine. Gelatinous precipitate formed with 1:1000 of reagent.
2. Wagner's reagent: a reddish brown precipitate formed before crystallization takes place.
3. yellow brown shiny crystals are formed with zinc chlor-iodide
4. Alkaloids form a needle in 1:50 solution of potassium iodide.

Test for Nicotine

1. It forms an amorphous precipitate with Kraut's, Wagner's Mayer's reagent.
2. Small crystals are obtained with Barium chloride.
3. With more concentrated solution of Mercuric chloride forms an Amorphous precipitate.

Test for Quinoline

1. An Amorphous precipitate formed with Wagner's, kraut's and Mayer's reagent.
2. Light-pink color formed when 1:50 solution of Million's reagent added.

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The authors wish to acknowledge DST-SERB (DST-FTYS-SB/FT/CS-113/2013) for providing funding to carry out our research. Also, we wish to thank VIT University for providing infrastructure.

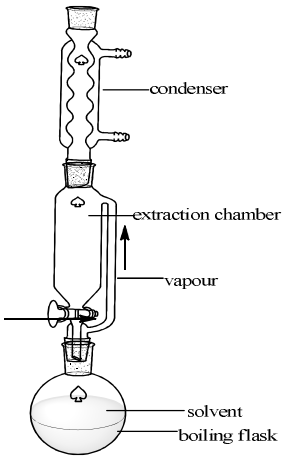

Fig 18: Soxhlet extraction

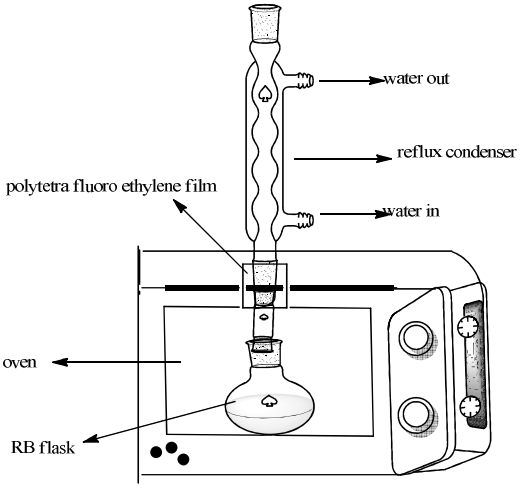

Fig 19: Microwave assisted extraction.

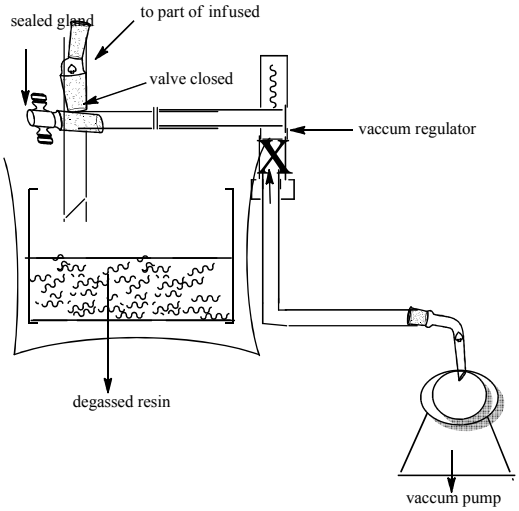

Fig 20: Ultrasonic assisted extraction

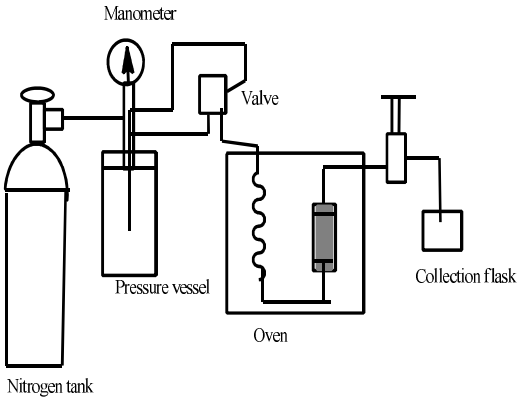

Fig 21: pressurized solvent extraction

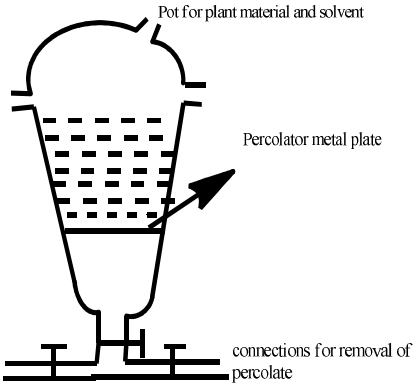

Fig 22: Percolation method

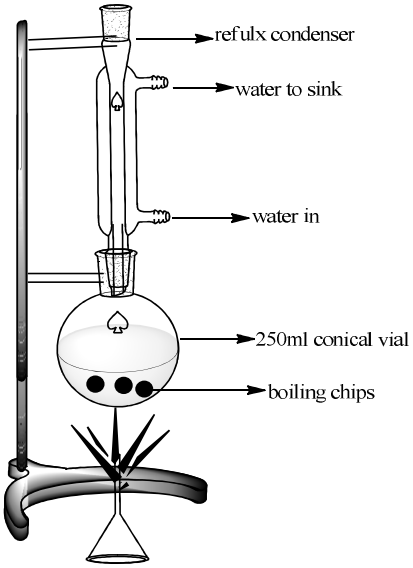

Figure 23: Heat reflux

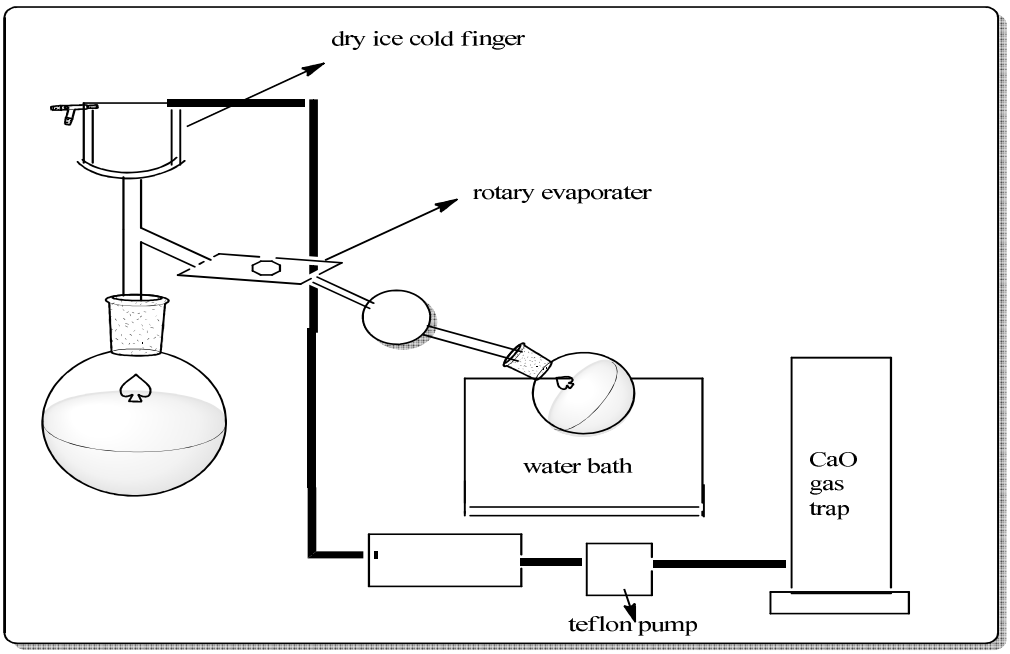

Fig 24: Rotatory evaporator

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