Ministry of Higher Education and Scientific Research
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Alkaloids

Total Synthesis & Extraction Techniques

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بسم الله الرحمن الرحيم

Dedication

This work is a fruit of countless and arduous sacrifices. Through the researchers' effort, this work is heartily and proudly dedicated to the people who serve as an inspiration. From parents and guardians, to classmates and circle of friends whom extended their help in the midst of problems while doing this work.

To the faculty and staff of Chemistry department and my supervisor. Above all, to our God Almighty who showered us His blessings in our everyday lives, especially for the strength, courage, patience, wisdom, time, and guidance in realization of this work.

The Researcher

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Outline:

Abstract

Alkaloid chemistry underlines the significance of the blocks, pathways and transamination reactions. The synthesis of the alkaloids is started from the acetate, shikimate, mevalonate and deoxyxylulose pathways. The main criterion for alkaloid precursor determination is the skeleton nucleus of the alkaloid. The following most important alkaloid nuclei exist: piperidine, indolizidine, quinolizidine, pyridon, pyrrolidine, imidazole, manzamine, quinazoline, quinoline, acridine, pyridine, sesquiterpene, phenyl, phenylpropyl, indole, -carboline, pyrroloindole, iboga, corynanthe and aspidosperma. Their synthesis occur in different pathways, which consist of a series of reactions and compounds as well as enzymes. The sequence of all reactions leading to any alkaloid synthesis is divided into precursor, intermedia, obligatory intermedia, second obligatory intermedia, alkaloid and its postcursors. The structural development of piperidine, indolizidine, quinolizidine, pyrrolizidine, izidine, pyrrolidine, tropane, imidazole, quinazoline, acridone, pyridine, sesquiterpene pyridine, phenyl and phenylpropyl, indole and manzamine alkaloids is presented in this chapter. Moreover, chemistry, biochemistry and molecular biology models of alkaloid biogenesis in organisms is discussed and method of alkaloid analysis described. Alkaloids are natural products. They can be isolated, detected and modified. Modification of alkaloids by chemical and biological processes and bioengineering can produce new applications. Chemistry not only investigates alkaloids, their structures and activities, but also develops methods for their structural manipulation.

Keywords: alkaloids, enzymes, genes, intermedia, metabolism, models, pathways, precursors, skeleton, synthesis

1-History and discovery of Alkaloids

The word "Alkaloid" was suggested in 1818 by K. FW. Meissner (1792-1853), a pharmacist in Halle, Germany. The word alkaloid is derived from the word alkali (regarding to basicity), from al gal (referring to soda) in Arabic. The -oid suffix, meaning like, obtained from the Greek. The definition of an alkaloid has changed significantly over the years, as more alkaloids have been structurally explained and the sources of alkaloids have expanded. (Shinji Funayama and Geoffrey A. Cordell, 2015). Alkaloid-containing plants have been utilized by humans since old times for restorative and recreational purposes. For instance, medicinal plants have been known within the Mesopotamia at slightest around 2000 BC. Human recognition of alkaloids is as old as civilization, since these substances have been used as drugs, in potions, medicines, teas, poultices, and poisons for 4000 years. It is likely that, in the hunt for food and in dealings with enemies, particular use was made of plants containing alkaloids for arrow poisons and this use probably preceded their medicinal use. Even today these poisons are still in use in Africa and South America. Arrow poisons have provided men with ouabain and k-strophanthin, for acute cardiac insufficiency, physostigmine for the treatment of glaucoma and myasthenia gravis, d-tubocurarine as a muscle relaxant in anesthesia, reserpine as an anti-hypertensive and psychotropic drug, and ajmaline in cases of cardiac rhythm disturbances. Sooner or later arrow poisons will disappear and there is a real need to continue to evaluate these poisons for active constituents. (Schmitz R., 1985) During the development of chemistry in the late 18^{th} century the first German pharmacist who discovered a method to extract alkaloid from a plant was young Friedrich Wilhelm Adam Serturner (1783–1841), He extracted crystal morphine from a seedpods of opium which is a highly addictive non-synthetic narcotic that is isolated from the poppy plant, Papaver somniferum (a drug that had been used for centuries for both its analgesic and narcotic properties) , First he tested it on stray dog and rat then he injected himself and 3 of his friends and the almost died due to overdose, (Schmitz R.،1985(was another 12 years before this lead was pursued. Between the years 1817 and 1820 the laboratory of Pelletier and Caventou at the Faculty of Pharmacy in Paris isolated so many active principles from crude drugs that even today no other laboratory has isolated as many active principles of pharmaceutical importance. Then at 1850 Scottish physician who invented syringe injected his wife with morphine, Later at 1874 German pharmaceutical company (Bayer) converted morphine into diacetyl morphine which known these days as Heroin. (Margaret F. Roberts and Michael Wink, 1998).

Figure.1 Bayer Company Heroin

In 1939 the number of alkaloids that had been isolated and structurally identified was on the order of 200. By 1989, the Dictionary of Alkaloids listed details of 10,000 alkaloids and new structures continue to be found. (Margaret F. Roberts and Michael Wink, 1998). In 1940s Quinine was used as a first antimalarial drug, It was isolated from cinchona bark herbs, It is still a useful drug for multidrug-resistant malaria. Also, in 1940s 4-aminoquinoline was developed as a synthetic drug from quinine, The drug was cheap and less toxic and it was best drug for malarial treatment for decades. Though after decades of use the drug was restricted for modern malarial therapy. Mefloquine is structurally related to quinine and was used as a treatment for chloroquine-resistant malaria, despite of the fact that it's use is limited because of resistance and neuropsychiatric side effect. (Wiesner, J. et al, M. 2003), Coniine, a polyketide-derived alkaloid, is toxic to humans and animals. It is a nicotinic acetylcholine receptor antagonist, which leads to prevention of the nervous system, eventually causing death by suffocation in mammals, Coniine is an alkaloid which is known to be present in a diversity of plants, including monocots, Conium and Sarracenia. Coniine's most famous victim was Socrates who was punished to death by poison chalice containing poison hemlock in 399 BC. In chemistry, coniine is the first alkaloid the chemical structure of which was established in (1881), and that was chemically developed in (1886). (Hotti, H., & Rischer, H, 2017), Nicotine is named after the tobacco plant Nicotiana tabacum, which in turn is named after Jean Nicot, French ambassador in Portugal, who sent tobacco and seeds from Brazil to Paris in 1560 and promoted their medicinal use. Nicotine was first isolated from the tobacco plant in 1828 by German chemists Posselt and Reimann. Its chemical empirical formula was defined by Melsens in 1843, and it was first manufactured by A. Pictet and Crepieux in 1893. Nicotine was originally banned for use in the United States but was brought in to the country for use as a horse tranquilizer then illegally moved to tobacco farms for use in their product. Nicotine is the principal alkaloid, which accounts for approximately 95% of the total alkaloids in tobacco.

Figure.2 Friedrich Sertürner, the German chemist who first isolated morphine from opium.

2- Introduction

Alkaloids are a diverse group of low molecular weight, nitrogen-containing compounds derived generally from amino acids. As secondary metabolites found in nearly 20% of plant species, alkaloids are supposed to play a defensive role against herbivores and pathogens. Owing to their potent biological activity, many of the nearly 12,000 known alkaloids have been founded as pharmaceuticals, stimulants, narcotics, and poisons. (Ziegler, J., & Facchini, P. J, 2008). Alkaloids are a group of molecules with a relatively large existence in nature around the Globe. They are very diverse chemicals and biomolecules, but they are all secondary compounds and they are resultant from the transamination process. Alkaloids are categorized according to the amino acids that afford their nitrogen atom and part of their skeleton. Similar alkaloids can have quite different biosynthetic pathways and different biological impacts. Alkaloids are derived from l-lysine, l-ornithine, l-tyrosine, l-tryptophan, l-histidine, l-phenylalanine, nicotinic acid, anthranilic acid or acetate. The terpenoid, steroid and purine alkaloids are also essential. For the biologist, the alkaloid is a pure and perfect natural product. From the biological point of view, the alkaloid is any biologically active and heterocyclic chemical compound which consist of nitrogen and may some pharmacological activity and, in many cases, medicinal or ecological use. This definition, as a relatively wide one based on application, can be criticized as inaccurate. However, it shows a general picture of what kinds of compound are under consideration. The biological and chemical nature of this group of compounds leads to the conclusion that each explanation of alkaloids is either too wide or too narrow. For the medical scientist, the term "alkaloids" means any group of nitrogenous substances of vegetable source, often of complex structure and high molecular mass. Moreover, it is important that alkaloids are usually heterocyclic, and may have primary, secondary or tertiary bases, or may contain quaternary ammonium groups. Certainly, the fact that alkaloids are only slightly soluble in water but soluble in ethanol, benzene, ether and chloroform is also really important, and highlighted in the medical definition. (Tadeusz Aniszewski, 2007). Alkaloids can react with acids and therefore creating salts, like inorganic alkalis. These nitrogen atoms can work like a base in acid-base reactions. In general alkaloids, which are treated as amines, the same as amines in their names, have suffix -ine. Alkaloids in pure form are generally colorless, odorless crystalline solids, but sometimes they can be yellowish liquids. Very often, they possess bitter taste. (Joanna Kurek, 2019).

S.No.	Alkaloid	mp (°C)	Optical rotation	Solubility
1.	Ajmaline	150-160	$[\alpha]_D^{20}$ + 144°	Chloroform, ether, ethanol, methanol
2.	Atropine	144-116	–	Benzene, chloroform, ether
3.	Berberine	145	–	Water
4.	Colchicine	142-150	$[\alpha]_D^{17}$ – 429°	Water, chloroform, ethanol
5.	Ephedrine	79	–	Water, ethanol, ether, chloroform, oils
6.	Hyoscyamine	108.5	$[\alpha]_D^{20}$ – 21.0°	Ethanol, dilute acids
7.	Morphine	197	$[\alpha]_D^{25}$ – 132°	Sparingly soluble in ethanol, chloroform, amyl alcohol,
8.	Physostigmine	105-106	$[\alpha]_D^{25}$ – 120°	Benzene, chloroform, oils
9.	Quinine	177	$[\alpha]_D^{17}$ – 117°	Chloroform, ether
10.	Reserpine	264-265 (dec.)	$[\alpha]_D^{23}$ – 118°	Chloroform, ethyl acetate, benzene.
11.	Strychnine	275-285	$[\alpha]_D^{18}$ – 104.3°	Chloroform, methanol, benzene
12.	Taxol	213-216 (dec.)	$[\alpha]_D^{20}$ – 49°	Methanol
13.	Vinblastine	211-216	$[\alpha]_D^{20}$ + 42°	Chloroform, ethanol
14.	Yohimbine	234	$[\alpha]_D^{20}$ + 108°	Chloroform, ethanol, benzene

Table.1 Solubility of some Alkaloids

Alkaloids shown quite various medicinal properties. Most of them have local anesthetic properties, but their practical use is narrow for clinical purpose. Morphine is designated as one of the most famous alkaloids which had been used and still is for medical purposes. This alkaloid is a powerful narcotic which is used as a pain-killer, but its usefulness is limited due to addictive properties, Methyl ether derivative of morphine (codeine) naturally occurring with morphine in the opium poppy, has an outstanding analgesic activity and is shown to be somewhat non-addictive. These alkaloids work as respiratory or cardiac stimulants. Next, the alkaloid which is used as medication in many clinical applications is atropine. For example, injection with atropine is used to cure bradycardia (low heart rate). Most of the alkaloids are elements of human diet, both in food and drinks. The plants in the human diet in which alkaloids are existing are not only coffee seeds (caffeine), cacao seeds (theobromine and caffeine), and tea leaves (theophylline, caffeine) but also tomatoes (tomatine) and potatoes (solanine). The most common alkaloid is caffeine which has also application as an ingredient of soft drinks like Coca-Cola to increase their taste and in drinks for active people who do exercises. Alkaloids stimulate human, for example, central nervous system, or directly affect the human brain. Nicotine is an alkaloid isolated from the tobacco plant (Nicotiana tabacum) and is a strong stimulant and the main ingredient in tobacco smoked in pipes, cigars, and cigarettes. This alkaloid is extremely addictive. Cocaine is a narcotic drug, which activity is not appropriate for medical purposes. This alkaloid has an opposite outcome than morphine. This compound produces in the human body a euphoric hyperarousal state, but high doses of it can lead to fibrillation and death. (Joanna Kurek, 2019), some alkaloids are illegal drugs and poisons. Poisonous activities of some alkaloids are known for ages. One of these is strychnine (from Strychnos class). One of the well-known poison curare (obtained from Chondrodendron tomentosum) used in the South Africa as narrow poison contains alkaloid tubocurarine. Coniine is an alkaloid obtained from Conium maculatum, which is an active ingredient of poison hemlock. Mescaline isolated from Anhalonium species has hallucinogenic affects. Psilocybin is a naturally occurring drug isolated from fungi classes Psilocybe mexicana and possesses psychedelic activity. During the past decades, many semisynthetic derivatives of naturally occurring alkaloids with several activities have been produced. Synthetic derivative of morphine is heroin, and, from lysergic acid naturally exist in C. purpurea, LSD was produced. Alkaloids are very essential compounds for human beings. For ages their extracts were used as treatment to rescue people from pain like morphine and some infections like quinine in malaria and colchicine in gout. Credits to alkaloids during ages, people can cure the diseases and progress their life. Scientists still keep trying to scheme and synthetize more and more semisynthetic and synthetic alkaloids derived from natural sources of alkaloids. These alkaloids possibly can possess interesting activities for clinical, pharmaceutical, synthetic, and many other useful properties. (Joanna Kurek, 2019).

Alkaloid	Action
Ajmaline	antiarrhythmic
Atropine, hyoscyamine, scopolamine	anticholinergic
Caffeine	adenosine receptor antagonist, stimulant
Codeine	analgesic, antitussive
Colchicine	remedy for gout
Emetine	emesis, antiprotozoal agent
Ergot alkaloids	vasoconstriction, Uterotonic, hallucinogenic
Morphine	analgesic
Nicotine	nicotinic acetylcholine receptor agonist, stimulant
Physostigmine	acetylcholinesterase inhibitor
Quinidine	antiarrhythmic
Quinine	antimalarial, antipyretic
Reserpine	antihypertensive
Tubocurarine	relax muscle
Vinblastine, vincristine	antitumor
Vincamine	antihypertensive, vasodilating
Yohimbine	aphrodisiac, stimulant

Table.2 Clinical properties of some Alkaloids

Alkaloids are an important element of the current cancer chemotherapy and are widely used as main compounds by medicinal chemistry researchers. Alkaloids with clinical uses affect the evolution of cell cycle by alteration of microtubule polymerization or inhibition of topoisomerases. However, it has been showed that an increasing number of alkaloids, including the classical anticancer alkaloids, can present numerous cellular targets, including mitochondria, affecting selectively to cancer cells. On the other hand, the severe toxicity and development of chemoresistance have driven to search strategies or new targets to enhance tumor selectivity. In this sense, selective targeting is currently an active research field, whose findings allow fully exploiting the pharmacological potential of this type of molecules. Furthermore, it offers the possibility to recover compounds discarded in previous pharmacologic studies. (Eduardo sobrazo-Sanchez, 2015).

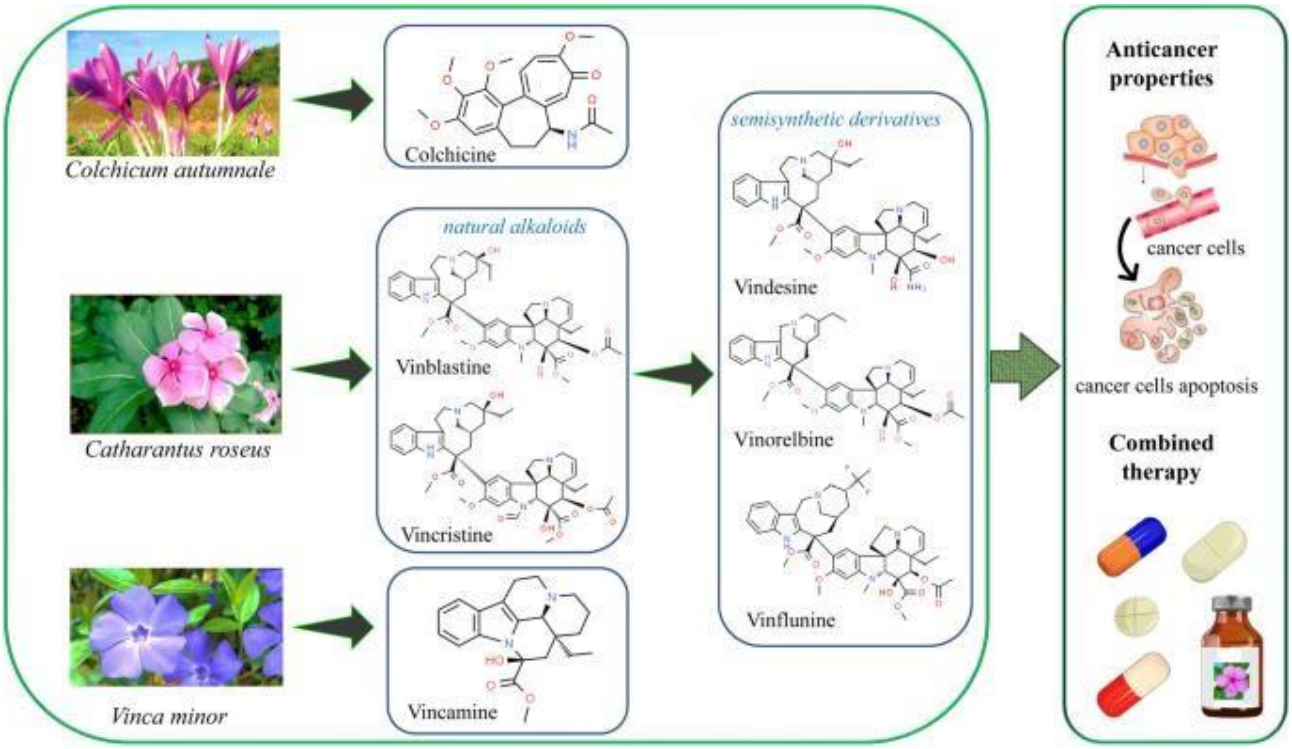


Figure.3 Alkaloids that are used as anti-cancer drugs

3- Classification of Alkaloids

3.1 Classification of Alkaloids upon Biogenesis

From a structural perception, alkaloids can be categorized, according to their molecular precursor, structures, and origins or on the biological pathways used to gain the molecule, there are three significant types of alkaloids: (1) True alkaloids, (2) Protoalkaloids, and (3) Pseudoalkaloids. True alkaloids and protoalkaloids are formed from amino acids, whereas Pseudoalkaloids are not derived from these compounds. (Dey, P., Kundu, A.,2020)

3.1.1 True Alkaloids

This type of alkaloids are derived from amino acids and they possess a nitrogen-containing heterocyclic ring. They are extremely reactive in nature and have strong biological activity, they form water-soluble salts, and most of them are crystalline in nature, which conjugates with acid and form a salt. Nearly all true alkaloids have bitter taste and solid, apart from nicotine, which is a brown liquid. Their existence in plants occurs in three forms: (a) in Free-state, (b) as N-oxide, or (c) as salts. Various amino acids like L-phenylalanine/L-tyrosine, L-ornithine, L-histidine, L-lysine are the central sources of true alkaloids, Cocaine, morphine, quinine are the common true alkaloids found in nature.(Dey, P., Kundu, A.,2020)

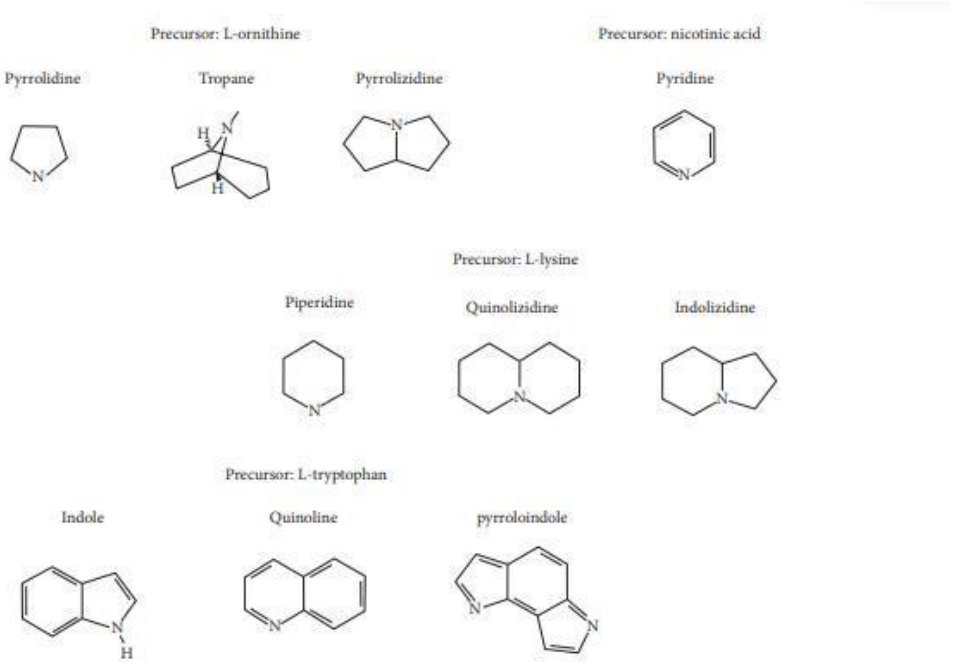


Figure.4 Structures of True alkaloids

3.1.2 Protoalkaloids

This type of alkaloids consist of nitrogen atom, which is derived from an amino acid but is not part of the heterocyclic ring system. L-Tryptophan and L-tyrosine are the main precursors of this type of alkaloids. This minor group is structurally composed of simple alkaloids. Yohimbine, mescaline, and hordenine are the main alkaloids of this type. They are used in many health disorders, including mental illness, pain, and neuralgia.(Dey, P., Kundu, A.,2020)

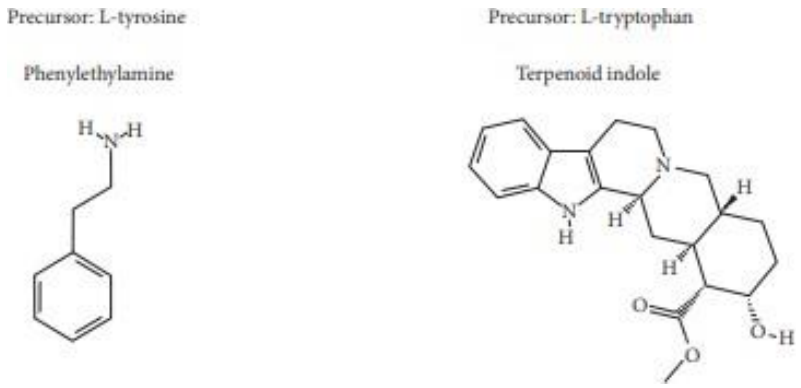


Figure.5 Structures of Protoalkaloids

3.1.3 Pseudoalkaloids

The basic carbon skeleton of pseudoalkaloids is not directly derived from amino acids; instead, they are linked with amino acid pathways where they are derived from by amination or transamination reaction from forerunners or postcursors of amino acid, Nonamino-acid precursors can also create pseudoalkaloids. They can be phenylalanine or acetate derived. Capsaicin, caffeine, ephedrine are very common examples of pseudoalkaloids. (Dey, P., Kundu, A.,2020)

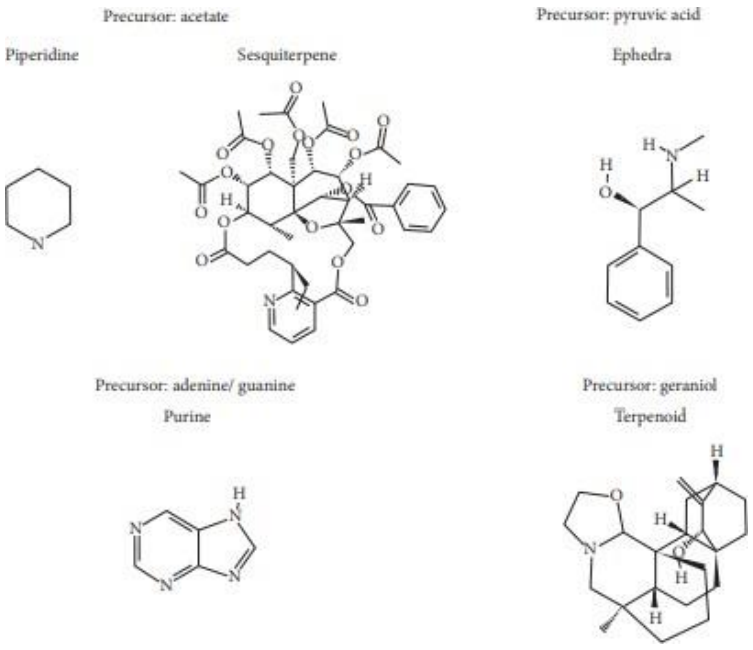


Figure.6 Structures of Pseudoalkaloids

3.2 Classification of Alkaloids according to their ring structure:

1. Pyrrol and pyrrolidine.
2. Pyrrolizidine.
3. Pyridine and piperidine.
4. Tropine.
5. Quinolone.
6. Isoquinolone.
7. Aporphine.
8. Norlupinane.
9. Indole.
10. Indolizidine.
11. Imidazole.
12. Purine.
13. Steroids.
14. Terpenoids

(Dey, P., Kundu, A., 2020)

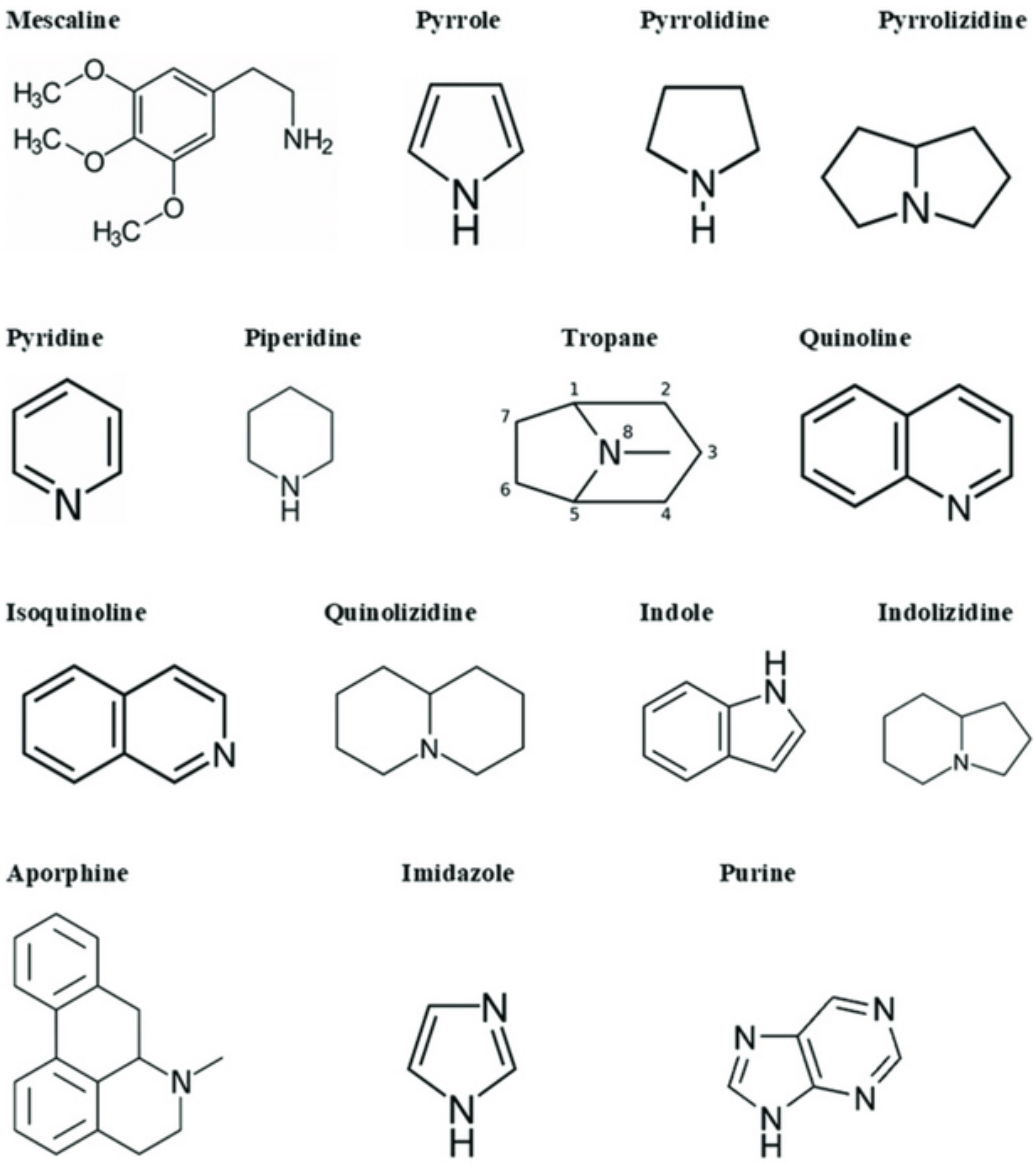


Figure.7 Ring Structures of Alkaloids

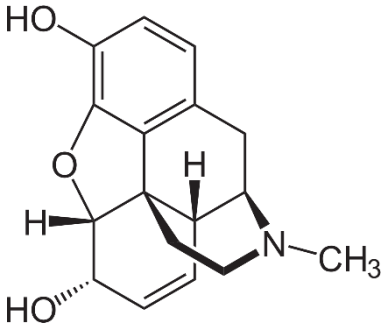
4-Most Common Alkaloids and their Total synthesis:

4.1-Morphine

Morphine is a pain medication which originate naturally in a number of plants and animals. It has direct effects on the central nervous system to reduce the feeling of pain. It can be taken for both acute pain and chronic pain either by mouth, being injected into a muscle or under the skin, intravenously, into the space around the spinal cord, or rectally. In 2013, nearly 523 tons of morphine was isolated and approximately 45 tons were directly taken for pain. About 70 percent of morphine is used to make other opioids such as hydromorphone, oxymorphoneand heroin. Morphine has also been used traditionally in the curing of acute pulmonary edema, although there is slight evidence to support this practice. 10 Morphine is useful in reducing the symptom of shortness of breath due to both cancer and non-cancer reasons. Low dosage sustained-release of morphine significantly reduces breathlessness and minimal exertion from conditions such as advanced cancer or end-stage cardiorespiratory diseases. (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019)

4.1.1-Side effects of Morphine

Antagonistic effects of morphine include constipation by decreasing gut motility. Clinical studies consistently conclude that morphine, like other opioids, often causes hypogonadism; a condition that causes poor functioning of testes in men and ovaries in women and also accountable for hormone imbalances in chronic users of both sexes. This side effect morphine is dose-dependent and take place in both therapeutic and recreational users. Morphine can interfere with menstruation in women by suppressing levels of luteinizing hormone. One of the big threats of morphine use is that addiction to the drug develops quickly. An addiction to the drug means that the user needs more and more of it to feel the same effects formerly felt from a smaller dose. As higher and higher doses are regularly used, the chances of a lethal overdose increase. Additionally, morphine activates the brain's (pleasure centers), which means that taking the drug is often considered highly enjoyable by the addict person, making him or her to focus all energies and efforts on securing more morphine. (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019)

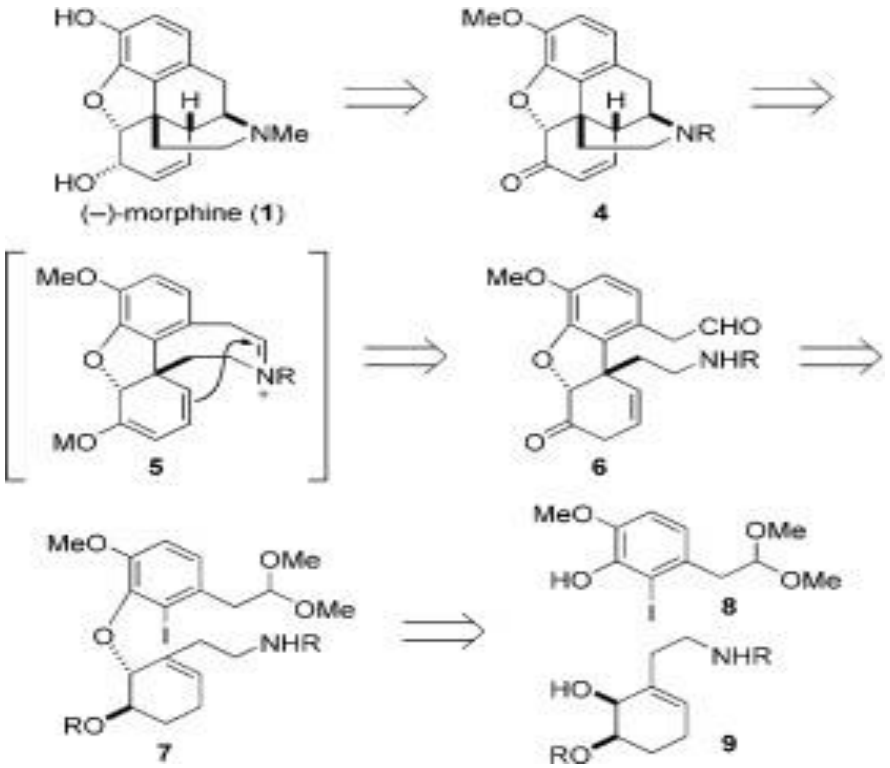


Structure.1

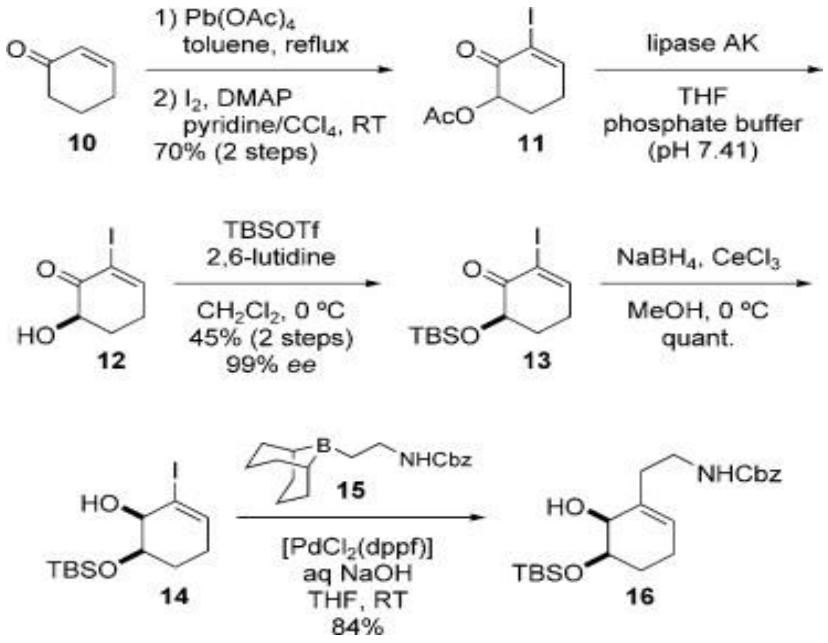
Structure of morphine

4.1.2-Total Synthesis of Morphine

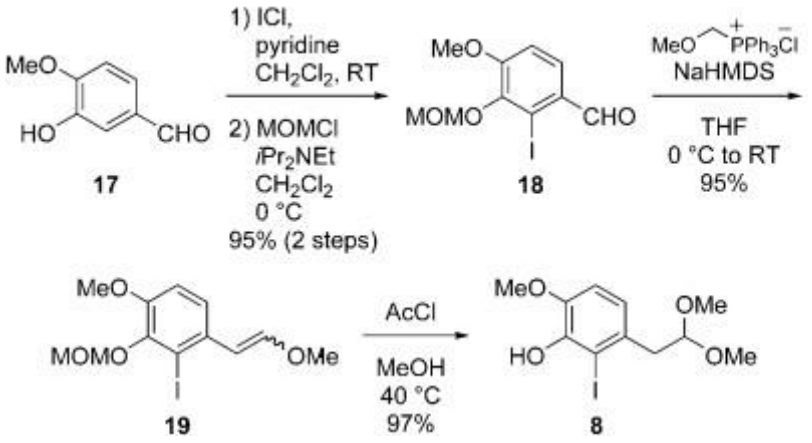
We have advanced an well-organized total synthesis of (--)-morphine in 5% overall yield with the longest linear sequence consisting of 17 steps from 2-cyclohexen-1-one. (Koizumi, H., Yokoshima, S., 2010)



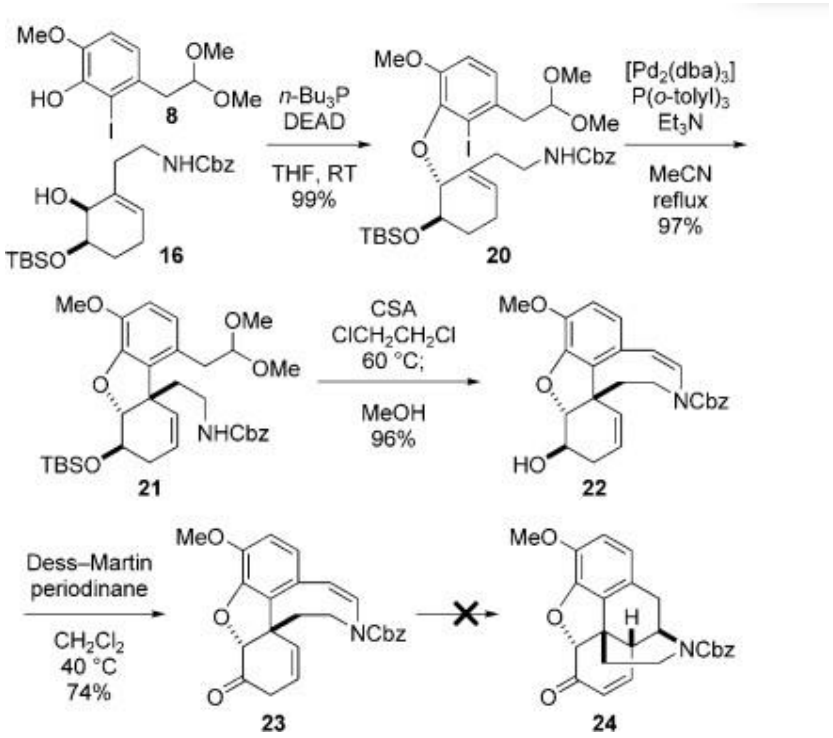
Scheme 1. Retrosynthesis



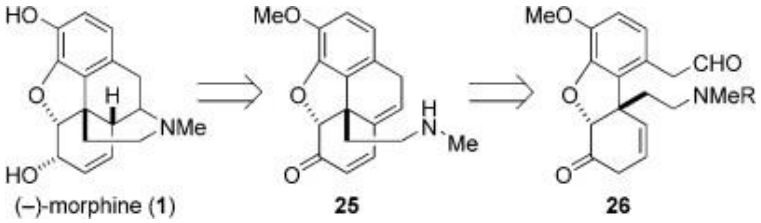
Scheme.2 Preparation of cyclohexenol 16. DMAP=4-(dimethylamino)- pyridine, Cbz=benzyloxycarbonyl, THF=tetrahydrofuran, TBS=tertbutyldimethylsilyl, dppf=1,1-bis(diphenylphosphino)ferrocene.



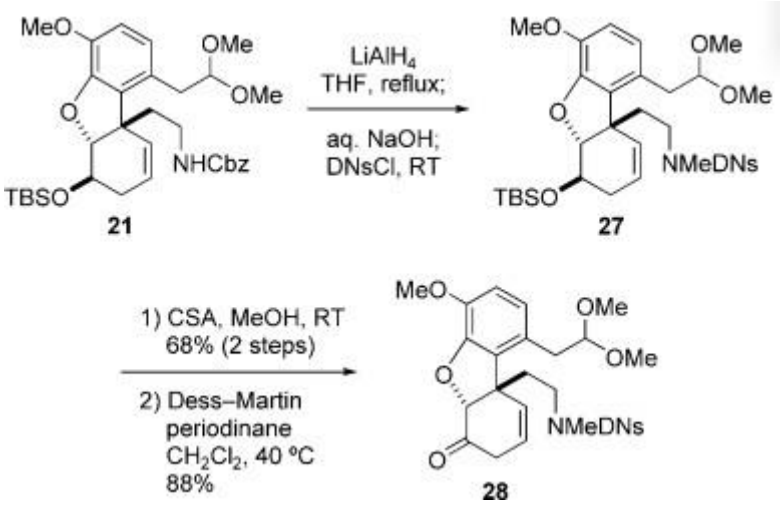
Scheme.3 Preparation of phenol unit 8. MOM=methoxymethyl, NaHMDS=sodium bis(trimethylsilyl) amide.



Scheme.4 Attempted Mannich-type reaction. DEAD=diethyl azodicarboxylate, dba=(E,E)-dibenzylideneacetone, CSA=camphorsulfonic acid



Scheme.5 Alternative retrosynthesis.



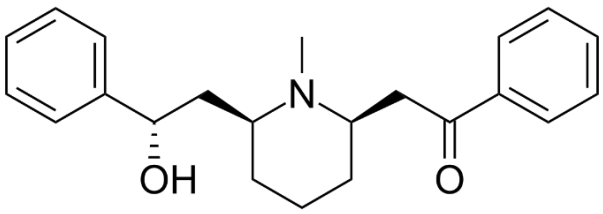
Scheme.6 Preparation of key intermediate 28. DNsCl=2,4-dinitrobenzenesulfonyl chloride

4.2-Lobeline

Lobeline is the derivative of plant Lobelia inflata (Indian tobacco). It is both an agonist and an antagonist at nicotinic receptors, although it is not structurally connected to nicotine. It prevents nicotine- and amphetamine-induced dopamine release by interacting with the tetrabenazine-binding site on the monoamine transporter. It also prevents dopamine re-uptake. It has been used in smoking cessation, but is ineffective.

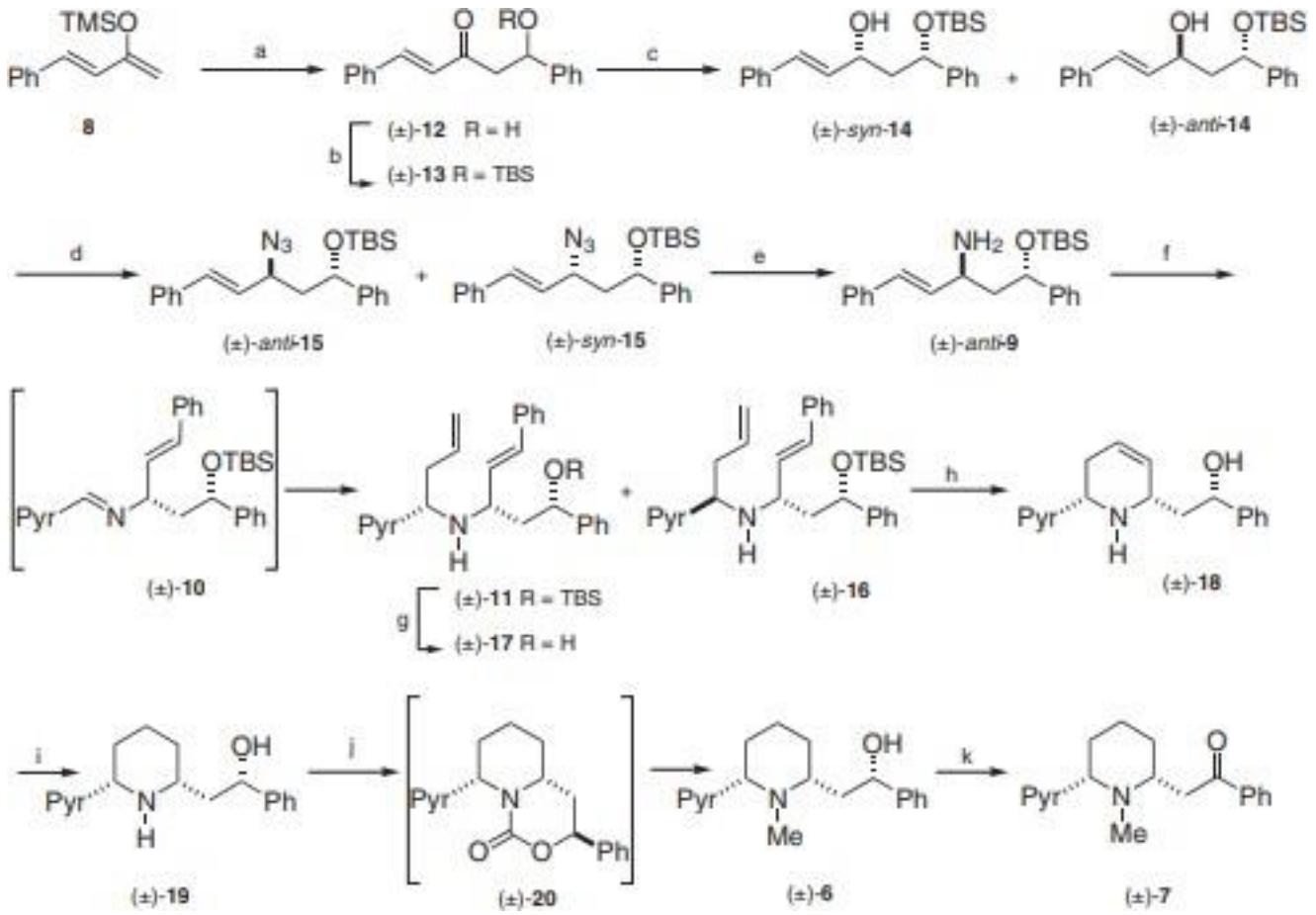
4.2.1-Side effects of Lobeline

Nausea, vomiting, coughing, tremor, and dizziness have been noticed with an average dose of lobeline. It can also cause nausea, sweating, and palpitation when inhaled from a cigarette.（Dwoskin, L. P., & Crooks, P. A. 2002)



Structure.2 Structure of Lobeline

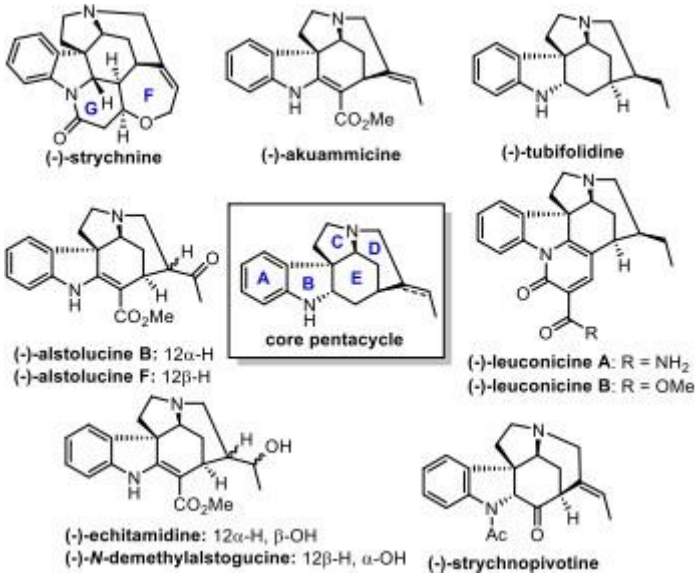
4.2.2-Total Synthesis of Lobeline



Scheme.7 Reagents and conditions: (a) PhCHO, $BF_3 \cdot OEt_2$, CH_2Cl_2, –78 °C, 1h (91%); (b) TBSCl, Et3N, DMAP, DMF, r.t., 8h (92%); (c) $LiAlH_4$, Et_2O–THF (9:1), –100 °C, 4 h (91%, ratio syn-14/anti-14 = 9:1); (d) DPPA, DBU, PhMe, r.t., 20 h (86%, ratio anti-15/syn-15 = 9:1); (e) Zn, NH_4Cl, EtOH–H_2O (3:1), reflux, 2 h (for anti-9 66% and for syn-diastereomer 12%); (f) 3-Pyr-CHO, $MgSO_4$, Et_2O, r.t., 7 h, then allyl MgBr, –78 °C to r.t., 4 h (85%, ratio 11/16 = 85:15); (g) TBAF, 0 °C to r.t., 7 h (82%); (h) Grubbs II, PTSA, CH_2Cl_2–t-BuOH (20:1), reflux, 24 h (67%); (i) H_2, Pd/C, EtOH, r.t., 48 h (83%); (j) NaH, CDI, THF, reflux, 3 h then LAH in excess, r.t., 12 h (82%); (k) ClCO–COCl, DMSO, CH_2Cl_2, –78 °C, then (±)-6, 15 min, Et_3N, –78 °C to r.t. (95%).

4.3-Strychnos

Strychnos henningsii is a small evergreen tree or shrub that consist of leathery leaves, commonly distributed in East and South Africa, used for treatment of numerous ailments in ATM such as gynecological complaints, abdominal pain, snake bite, gastrointestinal pain, rheumatism, malaria, and diabetes mellitus.



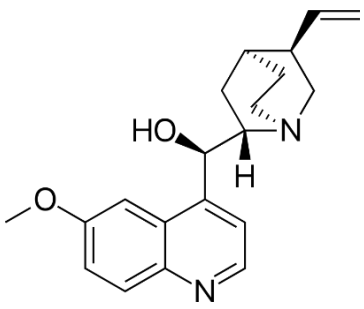
Structure.3 Representative strychnos alkaloids

4.4-Quinine

Malaria is defined as one of the most common infectious diseases in the world. Almost 1 million deaths of mostly children were caused by malaria. Now, there are over 100 countries fighting with malaria, of which 45 of these countries are within African territory. In spite of the fact that malaria is treatable and avoidable, its occurrence increased in the 1980s and 1990s as the malaria parasites developed resistance to the commonly used malaria drugs. Quinine and other cinchona alkaloids including quinidine, cinchonine and cinchonidine are effective against malaria, Quinine has an instant action against intra-erythrocytic malaria parasites. It is rapidly absorbed both orally and intravenously, reaching peak concentration within few hours. . (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019)

4.4.1-Side effects of quinine

Quinine has a low therapeutic index, and adverse effects with its use are considerable. The side effects commonly seen at therapeutic concentrations are referred to as cinchonism, with minor forms including tinnitus, slight impairment of hearing, headache and nausea. . (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019)



Structure.4

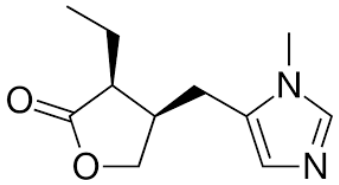
Structure of quinine

4.5-Pilocarpine

Pilocarpine is a naturally occurring alkaloid derived from the leaves of South American plants of the genus Pilocarpusand commercial production is derived entirely from the leaves of Pilocarpus microphyllus. It cause the stimulation of secretion of large amounts of saliva and sweat and it is used to cure dry mouth (xerostomia), mainly in Sjögren's syndrome, Pilocarpine is presented in both tablet and capsule formulation as well as a liquid-based preparation. It can increase secretion by the exocrine glands, including the sweat, salivary, lacrimal, gastric, pancreatic and intestinal glands, and the mucous cells of the respiratory tract. Also, pilocarpine increases smooth muscle tone and motility in the intestinal and urinary tracts, gallbladder, biliary ducts, and bronchi. . (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019)

4.5.1-Side effects of Pilocarpine

Most of the adverse effect of pilocarpine is linked to its non-selective action as a muscarinic receptor agonist. Pilocarpine has been known to cause excessive salivation, sweating,bronchial mucus secretion, bronchospasm, bradycar dia, vasodilation, and diarrhea. Eye drops can result in brow ache and chronic use in miosis. G.Systemic injection of pilocarpine can compromise the blood brain barrier letting pilocarpine to gain admission to the brain which can lead to chronic epilepsy. Epilepsy encouraged by injected pilocarpine has been used to progress animal models in rodents in order to study human epileps. (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019)



Structure.5

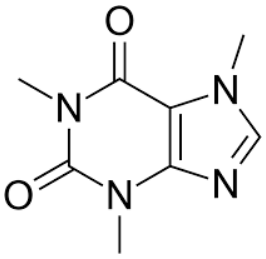
Structure of Pilocarpine

4.6-Caffeine

About sixty plant species are known to contain caffeine. Caffeine in plants acts as a natural pesticide: it paralyze and destroy predator insects feeding on the plant. High caffeine levels are mostly found in coffee seedlings when they are developing foliage and absence mechanical protection. Caffeine is the most widely consumed psychoactive drug in the world and one of the most widely studied ingredients in the food supply. Caffeine is generally used as pain reliever in medications such as Midol and Vanquis, which contains doses ranging from 33 to 60 mg. It is used therapeutically in combination with ergotamine to cure migraine headaches and in combination with non-steroidal anti-inflammatory analgesics. (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019)

4.6.1-Side effects of Caffeine

Whether or not caffeine which can result in an addictive disorder depends on how much caffeine is taken. Compulsive caffeine consumption under any conditions has not been witnessed, and caffeine is therefore is generally considered as non-addictive. Caffeine dependence can involve withdrawal symptoms such as fatigue, headache, irritability, depressed mood, reduced contentedness, inability to concentrate, sleepiness or drowsiness, stomach pain, and joint pain. Death from caffeine ingestion appears to be rare. (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019).



Structure.6

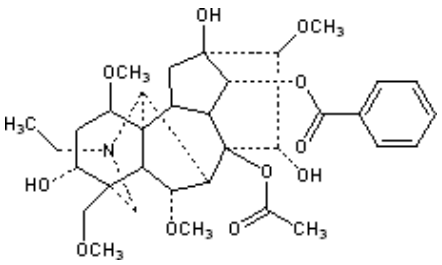
Structure of Caffeine

4.7-Aconitine

Aconitine is defined as alkaloid toxin formed by the Aconitum plant, it is also known as devil's helmet or monkshood. Monkshood is notorious for its toxic properties. In China, aconitine is also used in small doses as an analgesic and blood coagulant. The medicinal plant species of Aconitum are a rich source of alkaloids and flavanoids, many of which show wide spectrum of activity. The pharmacological analysis of Aconitum species and their compounds have shown several therapeutic effects. The key points of the scientific research have been the effects of the diterpene alkaloids on the central nervous system and the heart. (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019).

4.7.1-Side effects of Aconitine

The toxic properties of aconitine have been established in a variety of animals, including mammals dog, cat, guinea pig, mouse, rat and rabbit, frogs and pigeons. Counting on the way of exposure, the perceived toxic effects were: local anesthetic effect, diarrhea, convulsions, arrhythmias or death. According to a review of different reports of aconite poisoning in humans, hypotension, palpitations, chest pain , bradycardia, sinus tachycardia, ventricular ectopics and other arrhythmias, ventricular arrhythmias, nausea, vomiting, abdominal pain, and diarrhea are the main side-effects of aconitine. .(Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019).



Structure.7

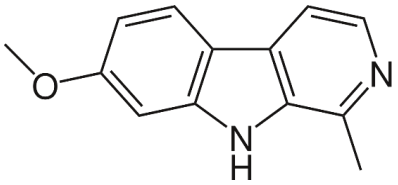
Structure of aconitine

4.8-Harmine

The use of harmine as a multi-purpose traditional medicine has been transformed into various commercial applications and it is an extremely valued phytoconstituent in the natural health, food and research area. Harmine has numerous traditional medicinal uses and pharmacological activity such as antimicrobial, anti-HIV and antiparasitic properties. Scientific studies conducted and confirmed many of the traditional uses including anti-inflammatory, antimicrobial, anti-parasitic and anti-cancer effects. Harmine has the capacity to stimulate dopamine release in the brain. Dopamine is a neurotransmitter responsible for transporting signals to the nerve cells. . (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019).

4.8.1-Side effects of Harmine

Oral or intravenous harmine doses ranging from 30–300 mg have caused agitation, bradycardia or tachycardia, blurred vision, hypotension, paresthesias and hallucinations. It has lately been shown in the Journal of Photochemistry and Photobiology that beta-carboline alkaloids such as harmine, bind with DNA and also display anti-tumor properties. . (Hamzat, Temitope Adejoke, Louis, Hitler,et al, 2019).



Structure.8

Structure of Harmine

4.9-Cocaine

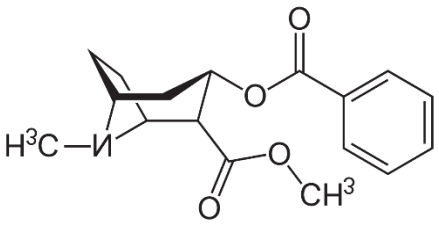
Cocaine "benzoylmethylecgonine" is an alkaloid isolated from the leaf of the Erythroxylon coca bush, which grows mainly in South America. It is obtainable in two forms: the hydrochloride salt and the "free base." Cocaine hydrochloride is prepared by dissolving the alkaloid in hydrochloric acid to form a water-soluble powder or granule that decomposes when heated. It can be taken orally, intravenously, or intranasally, the slang terms for which are "chewing," "mainlining," and "snorting," respectively. The free-base form is produced by processing the cocaine with ammonia or sodium bicarbonate (baking soda) to remove the hydrochloride. This formula is heat-stable and melts at 98°C, which allows it to be smoked. It is known as "crack" due to the popping sound it creates when heated. *(*Hamzat T. Adejoke, et al*, 2019)*

4.9.1-Side effects of Cocaine

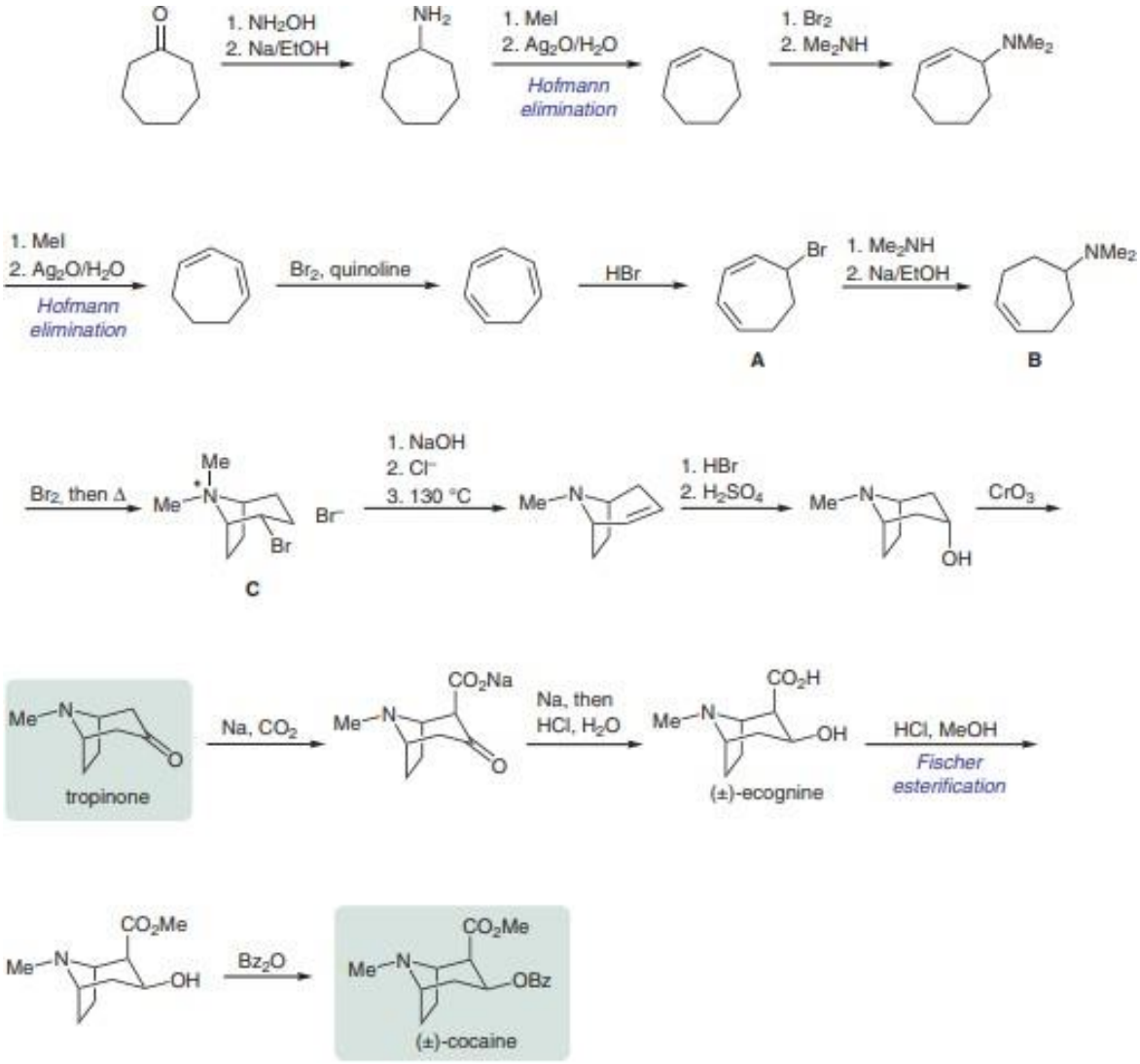
Since cocaine hydrochloride is well absorbed through all mucous membranes, abusers may be exposed a high blood concentration by means of intranasal, sublingual, intravaginal, or rectal administration, As compared with the intravenous injection of cocaine, the mucosal administration of the drug consequences in a slower onset of action, a later peak effect, and a longer period of action. Euphoria takes place within seconds after crack cocaine is used and is short-lived. Crack cocaine is considered to be the most powerful and addictive form of the drug. (Lange, R. A., & Hillis, L. D, 2001)

Structure.9

Structure of Cocaine



4.9.2-Total synthesis of Cocaine



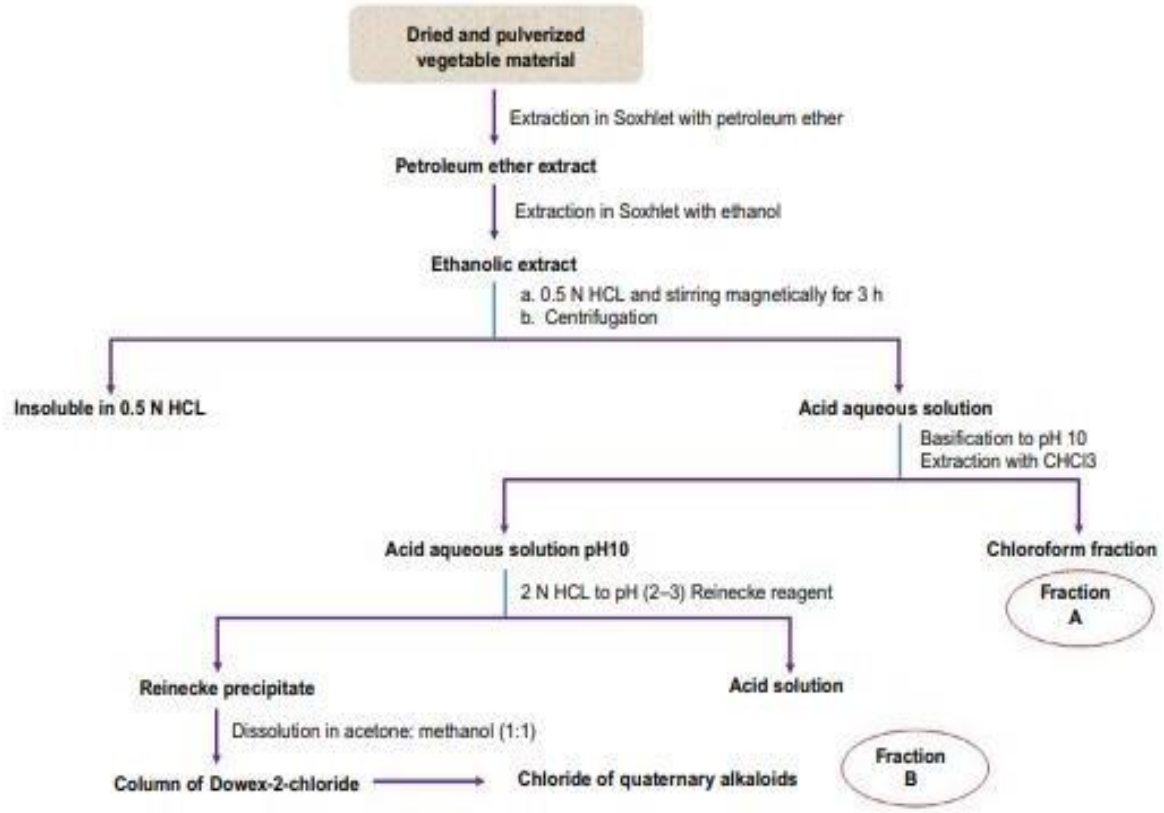


Figure.12 Extraction and purification techniques of indole alkaloids.

5.3-Liquid-liquid and solid-liquid extractions

If the precursor is liquid in nature, the partition method take place, where the distribution coefficient between the liquid form and solvent is considerable. This is an example of liquid-liquid extraction. In the case of extraction from solids, there are numerous subtypes of solid-liquid extraction.

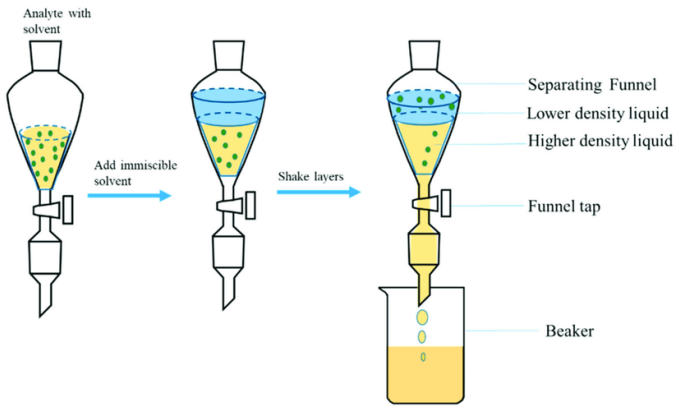


Figure.13 Liquid-Liquid Extraction

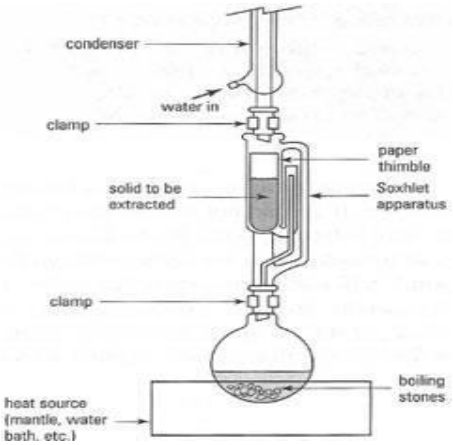


Figure.14 Solid-Liquid Extraction

5.4-Maceration

In this technique powder or bulk, plant compound is transported in a stoppered container and covered with a solvent for a certain time until the solubilized part is dissolved in the solvent. It is an example of the cold extraction process.

Figure.15 Maceration Extraction

5.5-Percolation

Indole-, isoquinoline-, and tropane-derived plant products are transported in a percolation tube plugged with cotton with a filter. For maceration, the solvent is placed in the plant material. The overall experiment is performed at room temperature. The extract along with extracted solvent is collected by a stopper at bellow. The process is sustained until the proper evaporation for the last residue of the solvent from the percolator.

Figure.11 describes the isolation techniques of Datura alkaloids (Tropane alkaloids).

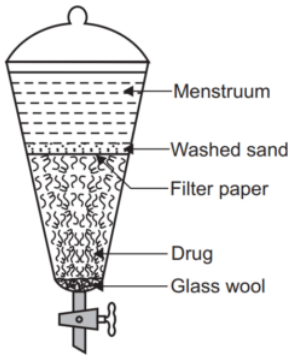


Figure.16 Percolation

5.6-Digestion

In this technique, temperature around (40–60°C) is applied at the time of extraction. The process is appropriate for thermostable plant materials. Adjustment of method is done by mixing the plants products using magnetic stirrer and mechanical stirrer. The extract is filtered after 8–12 h, therefore the fresh solvent is added. The method is continuous until the extraction of desire solutes.

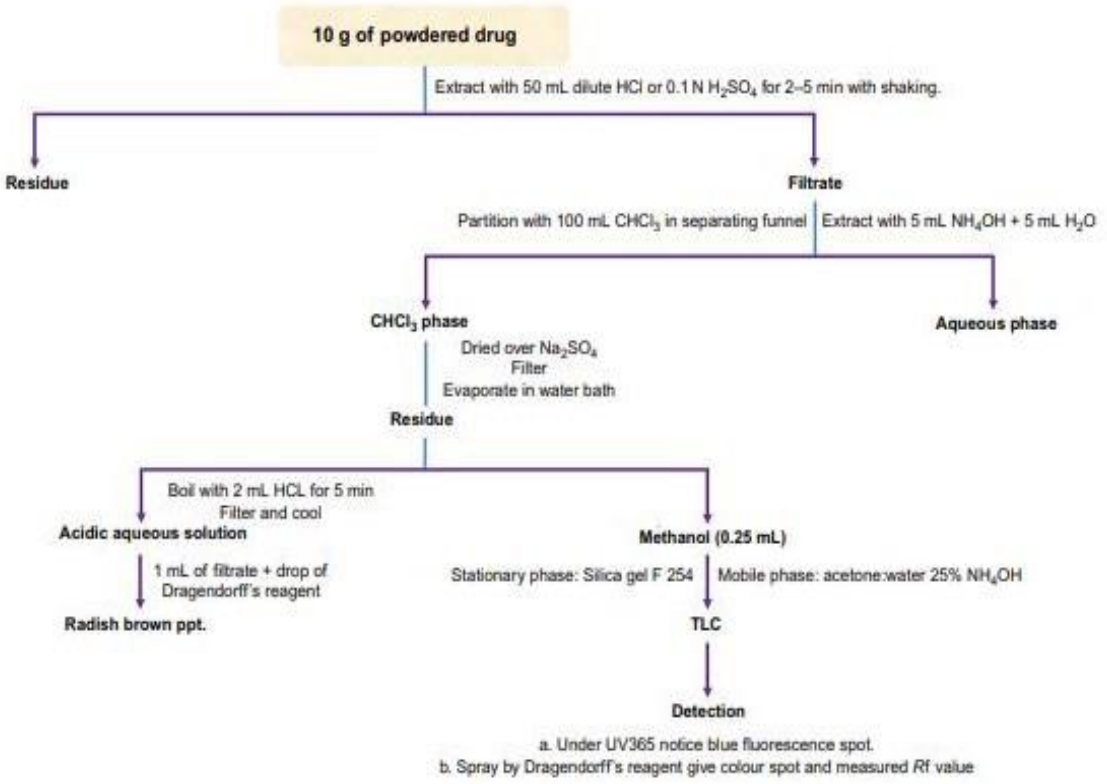


Figure.17 Extraction and purification techniques of datura alkaloids.

5.7-Infusion

In this technique, the plant material is extracted by using cold water or boiling water for a short time.

Figure .18 Infusion Extraction

5.8-Decoction

This process is suitable for extracting thermostable and water-soluble plant materials. Firstly the plant material is boiled in water, cooled, and strained.

Figure.19 Decoction

5.9-Extraction with Boiling Solvents (Reflexion)

In this technique, the plant material is kept with hot water. The solvent vapor is condensed by condenser fixed on top of the container and recycled Tincture Plant material extracted in presence of alcohol. Normally, ethyl alcohol is used at the ratio 1:5 Because of the alcohol content, the tinctures are kept in the closed system to evade decomposition.

5.10-Pressurized Liquid Extraction (PLE)

Another common name of this extraction technique is an accelerated solvent extraction (ASE) system or enhanced solvent extraction (ESE) system. In this method, temperature and pressure slowly increase. This raised temperature promotes the extraction process by increasing the diffusivity of the solvent, while increased pressure can promote the penetration process through matrix pore without changing the liquid state of organic solvent. The benefits of this extraction technique are a requirement of less solvent and less time.

Figure.20 PLE

5.11-Soxhlet Extraction

Soxhlet extraction has been used extensively for extracting valuable bioactive compounds from numerous natural sources. In this extraction technique, a slight amount of dry sample is placed in a thimble, which is employed in a distillation flask containing the solvent of particular interest. After reaching an overflow level, the solution of the thimble-holder is aspirated by a siphon, which drops the solution back into the distillation flask. This solution transports the extracted solutes into the bulk liquid. The solute remains behind in the distillation flask, and the solvent transport back to the solid bed of samples. The technique is repeated until complete extraction happens.

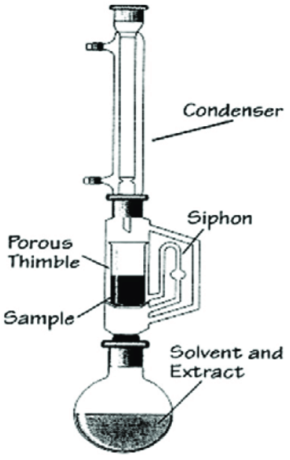


Figure.21 Soxhelt Extraction

5.12-Steam Distillation

The necessary oil is wxtracted from natural product through a steam distillation process. This process is very easy where vapors are made by steam, passing through the compounds. The steam volatile oil is recovered by condensation, where oil removed from the water.

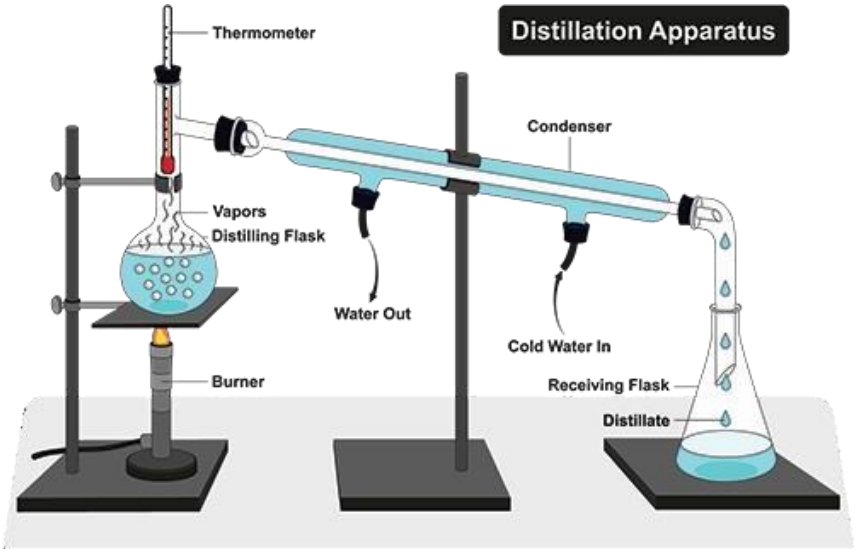


Figure.22 Steam Distillation

5.13-Hydrodistillation

Hydrodistillation process is a most common technique for isolation of essential oil. Plant products are soaked in water and boiled using a heating mantle. The important oil is separated out from the oil gland in the plant materials and transfer with the steam. Clevenger apparatus takes place in order to condense steam oil mixture and separate the oil part from the aqueous portion.

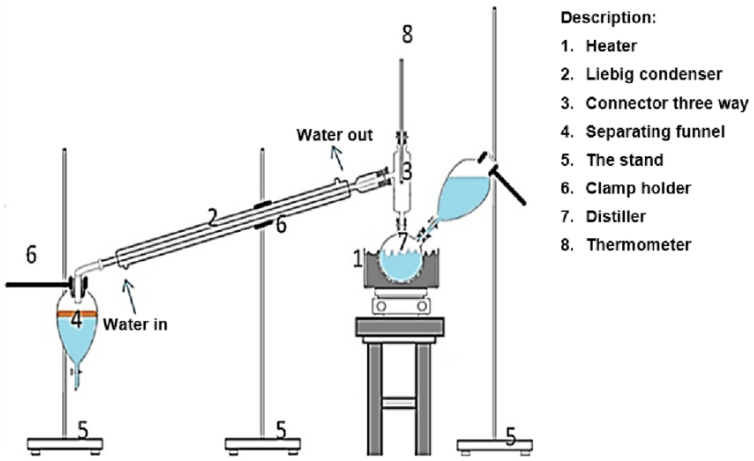


Figure.23 Hydrodistillation

5.14-Supercritical Fluid Extraction (SFE)

SFE is a diffusion-based process of splitting one component (extractant) from another (matrix) using supercritical fluids. Extraction circumstances usually are maintained above the critical pressure (Pc) and critical temperature (Tc) of the extraction solvent in use. The benefits of such an extraction technique is that it propose faster separation, higher selectivity, and better purity of the extract collected, Water and CO_2 are favored as extraction solvents for preparing herbal extracts by supercritical fluid extraction due to their ease in handling and nontoxic nature. Moreover, the use of modifiers, such as ethanol in varying percentages, is at times incorporated to accentuate the extraction efficiency by modification of polarities. SFE lately has been proven to be highly useful in preparing bioactive extracts from plant sources.

Figure.24 SFE

5.15-Ultrasonic Extraction

In this technique, high-frequency sound takes place to liberate the phytochemicals from the plant tissue. Ultrasound effect encourages the extraction used with mixtures of immiscible solvents. The main disadvantage of this technique is generating heat, which is dangerous to thermolabile products. To avoid such types of problem, extraction is carried on under an ice bath to decrease the temperature. This process is not appropriate for the isolation of large molecules like proteins or DNA.

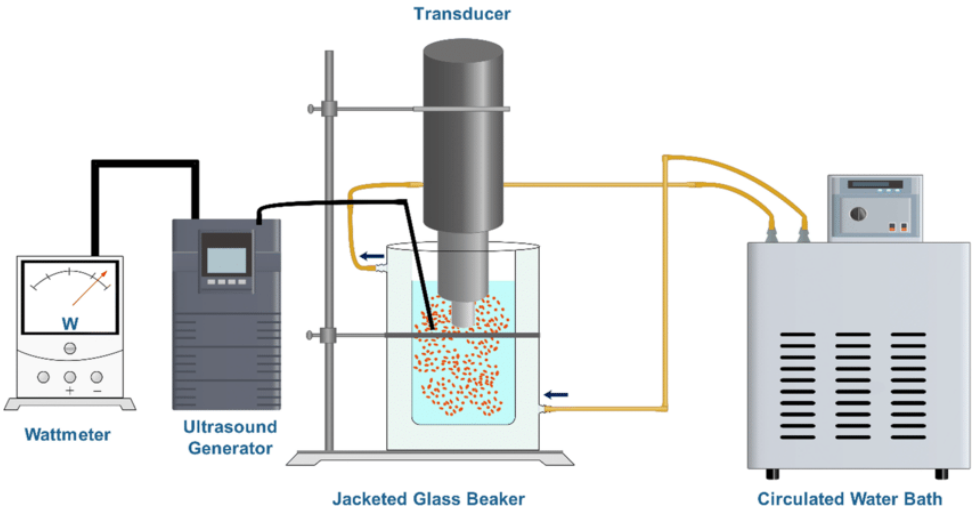


Figure.25 Ultrasonic Extraction

5.16-Microwave-Assisted Extraction (MAE)

Extraction methods engaging either diffused microwaves in closed systems or focused microwaves in open systems are being used now for their improved efficiency. Several types of microwave-assisted extraction methods are vacuum microwave-assisted extraction (VMAE), nitrogen-protected microwave assisted extraction (NPMAE), ultrasonic microwave-assisted extraction (UMAE), and dynamic microwave-assisted extraction (DMAE).

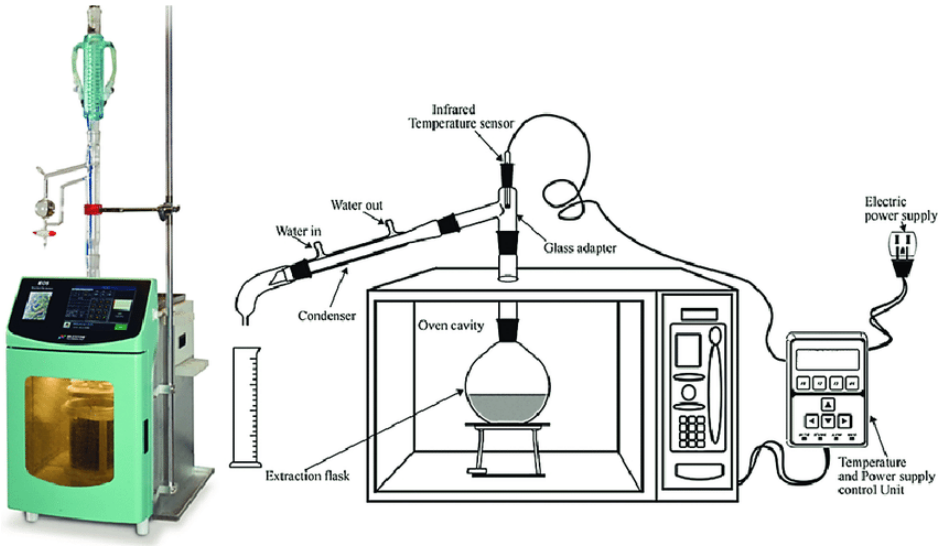


Figure.26 MAE

5.17-Solid-Phase Extraction

This type of extraction technique requires cartridges and disks with a variety of sorbents, where the solute molecules are particularly attached over the stationary phase surfaces. Normal phase, reverse phase, and ion exchange solid-phase extraction (SPE) units are existing. For example, polar compounds are eluted by using “Sep-Pak C18” cartridges (reverse phase), while the less polar compounds can be detached later.

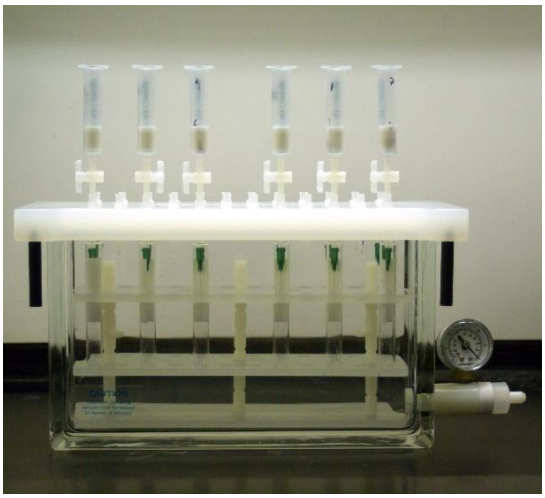


Figure.27 Solid-phase Extraction

5.18-Freeze Drying (Lyophilization)

Freeze drying is mostly used for concentrating the thermolabile substances such as proteins, antibiotics, and enzymes. The principle of lyophilization is that the aqueous solution is frozen and the ice is sublimed off to leave a dry residue. Initially, the material is cooled below its triple point. At the moment, temperature usually should continue at the range 50° C to 80°C. About 95% of the water in the compound is sublimated at the time of primary drying stage. The sublimed water vapor becomes condensed on a cold outward (50°C). In the secondary drying stage, residual water molecules are detached from the frozen material. Later removing water from the compound, nearby atmospheric temperature becomes equal. Finally, the residual water content reaches around 0.5%.

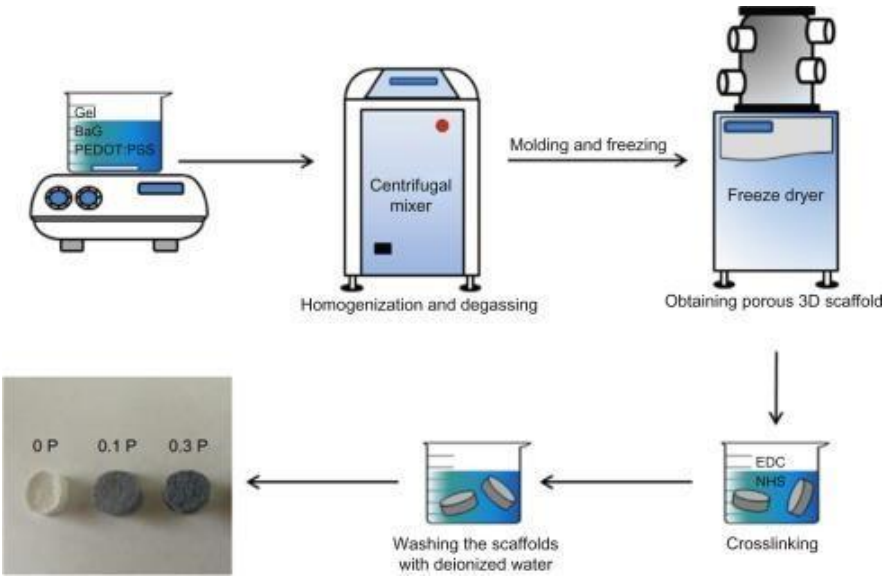


Figure.28 Freeze drying (lyophilization)

5.19-Protection of The Extract

Moisture, temperature, light, existence of oxygen and microorganisms are the essential key factors for proper storage of the indole-, isoquinoline-, and tropane-derived plant extracts. To evade water contamination, a suitable drying agent such as anhydrous sodium sulfate, calcium chloride, P_2O_5, $CuSO_4$, K_2CO_3, Sodium, CaO, $MgSO_4$ is used at the time of storage. Toluene is used as an antifungal agent in the aqueous extract. The extract should be stored in dark place for the protection against oxidation reaction. The extract should be transported in N_2 atmosphere to prevent decomposition. For prevention of decomposition, a reducing substance such as cysteine (1%, pH 7) is used and stored at cold temperature in the refrigerator or a deep freezer, and sealed hermetically.

5- Identification Tests for Alkaloids

The precipitation in Mayer, Wagner and Dragendorff identification confirm the presence of alkaloid compounds in the ethanol extract. The purpose of adding of sulphuric acid is because of the properties of alkaloid which is base. Therefore, it must be extracted in the acid solvents (Parbuntari, H., Prestica, Y., 2018).

a) Mayer's Test:

The positive result of Mayer test was confirmed by yellow precipitate. It was estimated as a complex of potassium-alkaloid. In the formation of Mayer reagent, the solution of mercury (II) chloride was added by potassium iodide and formed a red precipitate of Mercury (II) iodide. The excess of potassium iodide addition present to potassium tetraiodomercurate(II) formation. Alkaloids contain nitrogen atoms which consist of lone pair electrons. The lone pair electrons are examined to produce covalent coordinate bonding with metal ion. In alkaloid identification with Mayer reagent, the nitrogen in alkaloids was expected to react with metal ion of potassium (K^+) from potassium tetraiodomercurte(II) making a complex of potassium-alkaloid precipitating. The reaction was proposed as Figure 25.

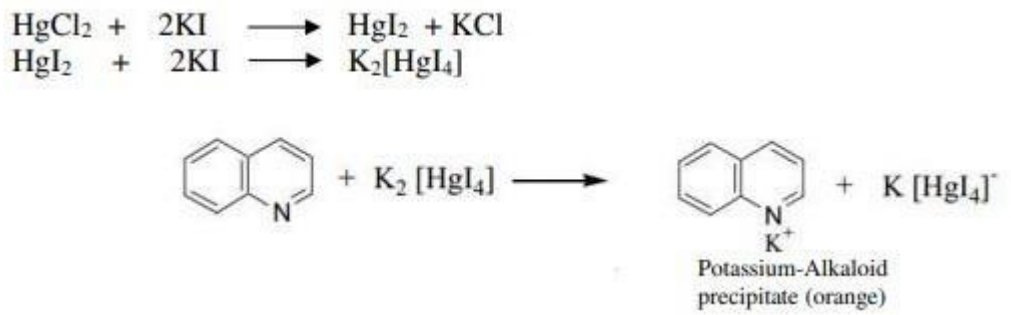


Figure.29 Mayer's reagent Reaction

b) Wagner's Test:

The positive results of alkaloid test in Wagner test was confirmed by the presence of brownish to yellowish precipitate. The precipitate was expected as the presence of potassium-alkaloid. In Wagner reagent preparation, iodine reacts with (I^-) ion from potassium iodide creating (I^{-3}) ion (brownish solution). In the Wagner test, the metal ion of (K^+) will bind as covalent coordinate bonding with nitrogen to alkaloid creating a complex precipitate of potassium-alkaloid. The reaction is predicted as Figure 26.

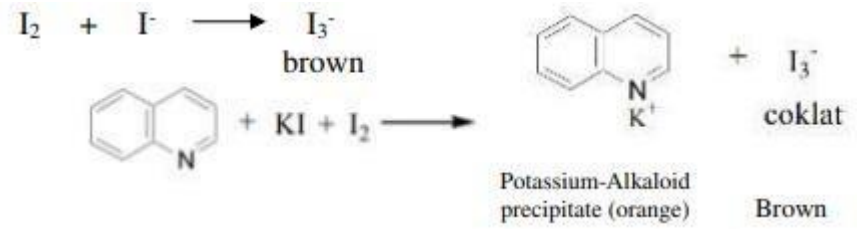


Figure.30 Wagner's reagent Reaction

c) Dragendorff's Test:

The positive result of alkaloid identification in Dragendorff test was identified as brownish or yellowish precipitate. The precipitate arises from complex compound of potassium-alkaloid. In Dragendorff reagent preparation, bismuth nitrate was dissolved in hydrochloric acid defending to hydrolisis reaction because salts of bismuth are easily hydrolised creating (BiO^+) ion. The reaction was proposed as Figure 27.

$$Bi^{3+} + H_2O \longrightarrow BiO^+ + 2H^+$$

The hydrolisis reaction will control the occurrence of (Bi^{3+}) ion in the solution. Therefore, the solution should be added an acid compound and moved the equilibrium to left. Furthermore, (Bi^{3+}) ion from bismuth nitrate $Bi(NO_3)_3$ may react to potassium iodide (KI) producing dark browninsh precipitate of Bismuth (III) iodide and dissolved in the excess of potassium iodide creating potassium tetraiodobismuthate. In the alkaloid identification of Dragendroff test, nitrogen proceeded to form covalent coordination bond with K+ ion (metal ion). The reaction is proposed as Figure 4.

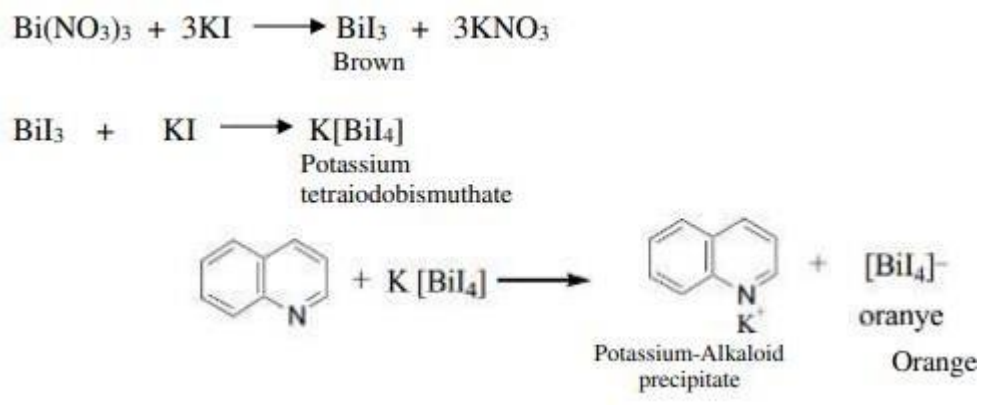


Figure.31 Dragendorff**'s** reagent Reaction

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