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Centre Universitaire de Guelma
Institut de Chimie Industrielle

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Thèse

Présentée au C. U. Guelma pour l'obtention du grade de:

MAGISTER

* * *

Spécialité: Chimie Industrielle
Option: Méthodes d'Analyses

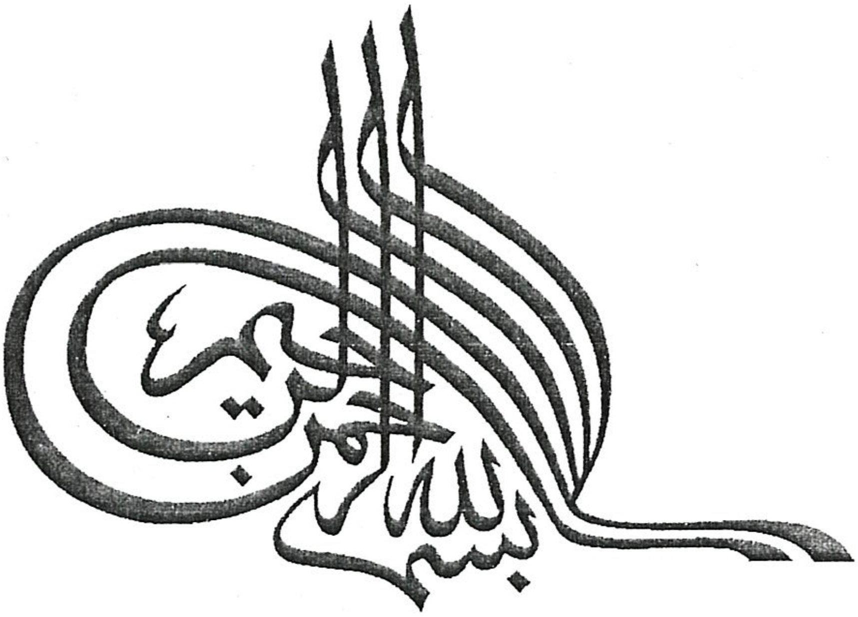
Par

Noureddine GHERRAF

Thème

INVESTIGATION OF ALKALOID CONSTITUENTS OF ALGERIAN PEGANUN HARMALA (ZYGOPHYLACEAE)

Membres de Jury:


بسم الله الرحمن الرحيم

« *Though this be madness yet there is method in't* »

W. Shakes Peare.

« *It must not be assumed that atoms of every sort can be linked in every variety of combination* ».

Titus Carus (100 - 55 B.C)

« *What is now proved was once only imagined* »

William Blake « The Marriage of Heaven and Hell »

« *What is written without effort is in general read without pleasure.* »

Samuel Johnson (1709 - 1784)

ACKNOWLEDGMENT

I would like gratefully to thank my supervisor for his invaluable assistance and guidance during the preparation of this project, as well as my teachers: Dr. Oumeddour R., Dr. Kadri M., Dr. Houache O. who have provided me the opportunity to continue my studies.

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I gratefully acknowledge the help and advice of chemical department of the university of Guelma especially Amar Lemouchi and Houria Bouchemala for their precious and continuous help as well as Messaouda who deserves my deep thanks.

I would like also to thank the jury members for they accepted to criticize this report and contribute to the discussion.

My gratitude which can not be put into words must go to the U.G.E.L members for their durable help throughout my stay in Guelma .

At last I thank my friends Ali Mellah, Hamed, Slimane, Haidar, for their help in the writing of this report .

The last but not the least to thank are all those who have contributed in the success of my academic carrier.

DEDICATION

TO MY PARENTS , BROTHERS AND SISTERS

STATEMENT

The experimental work in this thesis has been carried out by the author in the department of industrial chemistry of « Centre universitaire de Guelma » and « Centre universitaire de Ouargla » under the supervision of Doctor Djaballah Belkacemi .

This work has not been , and is not currently being presented for any other degree.

Centre Universitaire De Guelma
May 1997

ABSTRACT

Peganun Harmala is a famous plant widely used in traditional medicine in Algeria. It is used mainly to cure some diseases such as rheumatism and intestinal parasites .

This plant has not been studied in Algeria in spite of the importance it involves and the danger it presents when it is abused .Hence , this study is intended to make a person aware of the principal active ingredients of the plant both qualitatively and quantitatively and to develop simple and basic methods for any further investigation of the plant . This study consists of three parts :

The first parts deals with a literature review covering a general introduction and a listing of important types of alkaloids (history, nomenclature, pharmacology and general methods of separation).

The second part involves a physiological description and a taxonomic classification of Peganun Harmala in the plant kingdom .

The third part includes experimental work and deals with :

-A phytochemical screening of the different parts of the plant so as to check the presence of some important natural products (volatile oils, tannins, glycosides, saponins, cardenolides and steroids).

- Extraction, and isolation of the alkaloids using different procedures.

-Estimation of alkaloid content in different parts of the plant using different methods.

- Thin layer chromatography analysis.

- Gas liquid chromatography.

- Purification of alkaloidal mixture.

- Discussion and analysis of results.

We intend to pursue this research for other compounds whose presence has been confirmed by different chemical tests.

CONTENT

Appendix

I: GENERAL

1: INTRODUCTION:

The term ALKALOID (alkali-like) is applied to a large number of Nitrogen containing natural products most of them are of vegetable origin [1].

The major part of alkaloids are optically active and nearly all of them are of basic nature. Accordingly, they very often form salts with plant acids. Many of them have a bitter taste and quite a number of them possess curative properties. The function of alkaloids in plants is still a matter of controversy, they are regarded as by-products of plant metabolism, as reserve material for protein synthesis, as protective substances discouraging animal and insect attacks , as plant stimulants or regulators similar to hormones or simply as detoxification products.

Classification of alkaloids usually exclude simple amines, purines, betaines derived from aminoacids and other bases of biological importance, i.e. the primary metabolites which in contradistinction of the typical alkaloids, are not limited to one or a few species of plants. Nevertheless, the alkaloids make up the largest (over 4000 compounds isolated to date) [2] and most diverse of the groups of natural products. They occur most abundantly in the higher orders of the plant kingdom (they have been found in about 100 different families of flowering plants) and, so far as is known, they are virtually absent from the lower groups of plants with the exception of one or two families of the fungi. Moreover, they generally occur in plant tissue at points of intense cell activity (leaves, roots, bark and seeds) and they are generally found in living cells rather than in dead tissue.

2: HISTORY:

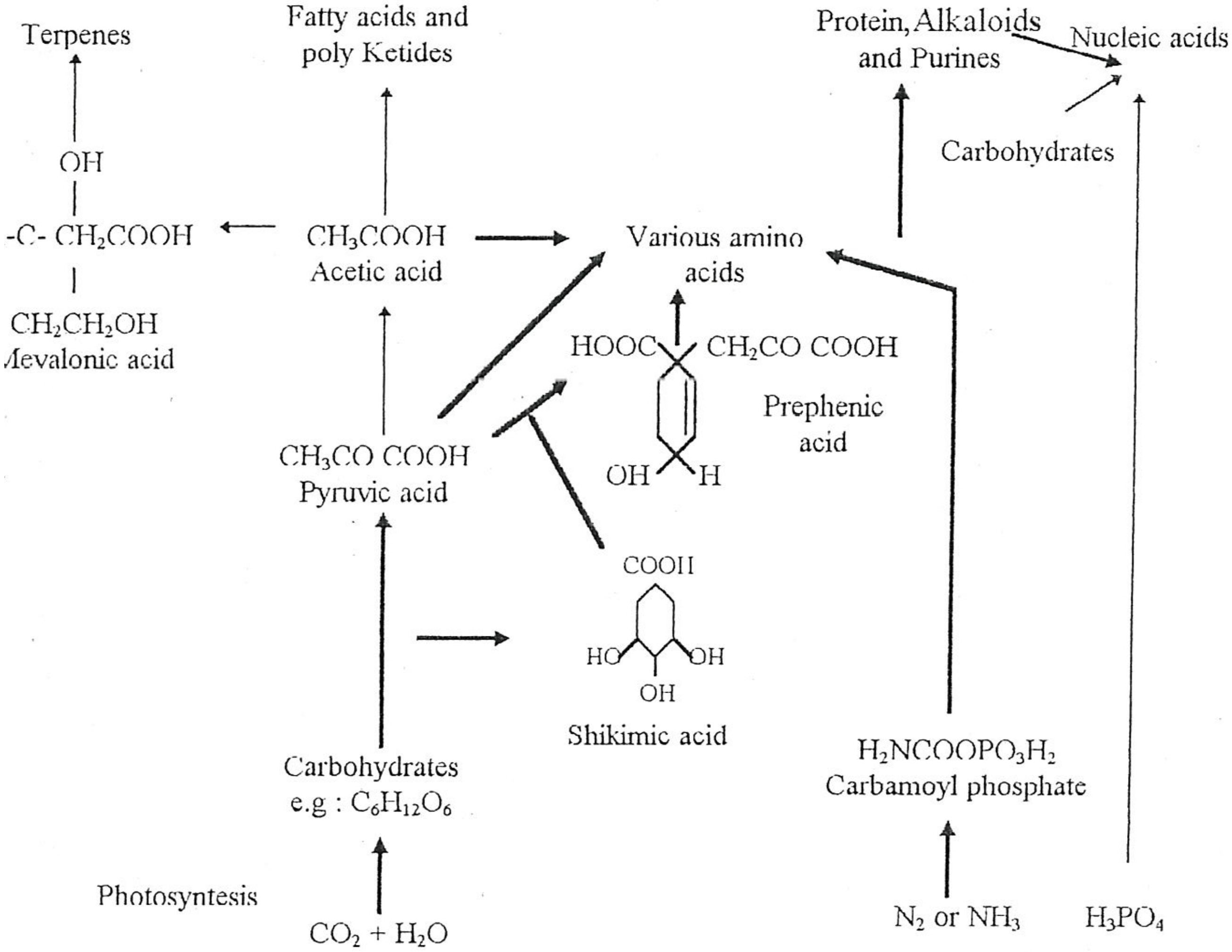
The history of alkaloids is almost as old as civilization. The mankind has used drugs containing alkaloids in potions, medicines, teas poultices, and poisons for 4000 years. Yet no attempts were made to isolate any of the therapeutically active ingredients from the crude drug until the early ninetieth century. Plant derivatives have been used from the remote antiquity to treat human diseases and preparations were described by the Egyptians as early as 1500 B.C. Chinese medicine, with its use of simple poly-pharmaceutical preparations called fongs, also utilized a wide variety of plants.

Advances in chemical technology permitted the isolations of the active principles from medicinal plants and a subsequent elucidation of their chemical structure.

Most of these pharmacologically active substances are alkaloids. Although there are large numbers of separate classes of alkaloids, indole derivatives of which more than 1400 are known are surprisingly well presented among the pharmacologically active compounds because of their molecular complexity . The majority of alkaloids precluded their structure during the nineteenth century or even the early twentieth century. By 1939 nearly 300 alkaloids had been isolated and about 200 of these had at least reasonably well defined structures. Up to 1950 more than 1000 alkaloids are noted.

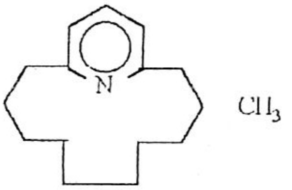
With the introduction of preparative chromatographic techniques and sophisticated spectroscopic instrumentation, the number of known alkaloids has risen dramatically. A review to the middle of 1973 counted 4959 alkaloids of which 3293 had known structures. By late 1978, the number stood at nearly 4000, structurally defined alkaloids. The main pathways in biosynthesis are presented in figure 1 .

Figure 01: The main pathways in biosynthesis. [1]

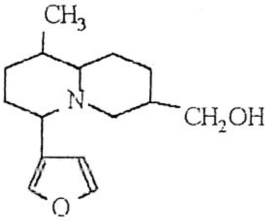


3: Occurrence:

The major source of alkaloids in the past has been the flowering plants. In recent years however there have been increasingly numerous examples of the occurrence of alkaloids in animals, insects, marine organisms, micro-organisms, and the lower plants. Some examples of this very diverse occurrence of alkaloids are the isolation of muscopyridine (1) from the musk deer, castoramine (2) from the Canadian beaver [3].



(1) Nuscopyridine

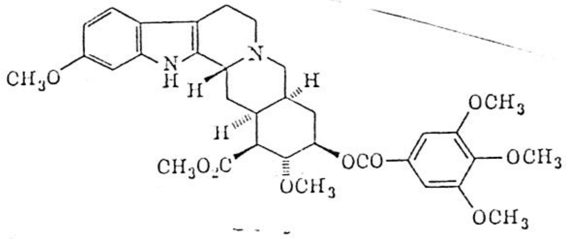


(2) Castoramine

The most important alkaloid containing families are the liliaceae, Amaryllidaceae, Solanaccac, compositac, rubiascac, loganiaccac, apocynaccac, Solanaccac and so forth.

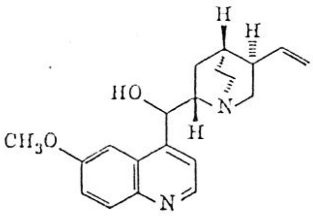
Within a given alkaloid - containing plant, the alkaloids may be highly localized in a particular plant part. For example reserpine (3) [2,4] is concentrated in the roots of Rauvolfia quinine (4) occurs in the bark, but not the leaves, and morphine (5) occurs in the latex of the Papaver Somniferum.

Even when the alkaloids do occur in a particular plant part, the range of concentration not only of the total alkaloids, but also of the alkaloids of pharmacological significance, may vary enormously. For example reserpine (3) may occur in concentration of up to 1% in the roots of Rauvalfia Serpentina.



Reserpine

(3)



Quinine

(4)

4: Nomenclature:

With so many different alkaloid types, a single unifying nomenclature is of course not possible. Even within a given group of alkaloids it is not often that there is a consistent system of nomenclature of numbering. One example is in the indole alkaloids where many different skeleta are found.

The only common characteristic of alkaloids names is that they terminate in « ine ». Beyond these alkaloids, like other natural products are given so- called (trivial) names. They may derive from the plant name.

e-g: Alalakine from .... Alalaka.

Cocaine from .... Erythoxylum Coca.

Atropine from .... Atropa Belladonna.

5: Classification:

It should be borne in mind that for alkaloids as a group of compounds, no single definition is all embracing. In place of this, numerous attempts have been made to provide a system of classification into which most alkaloids can be placed. The most widely accepted classification system groups of the alkaloids [25] are:

A: True Alkaloids.

B: Proto Alkaloids.

C: Pseudo alkaloids.

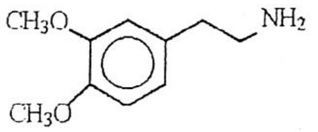
5-1: True Alkaloids:

The true alkaloids are toxic, they show a wide range of physiological activity. They are almost invariably basic. They normally contain nitrogen in a heterocyclic ring. They are derived from aminoacids. They are of limited taxonomic distribution and they normally occur in the plant as the salt of an organic or mineral acid.

5-2: Proto Alkaloids:

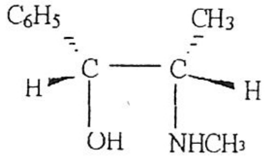
The proto alkaloids are relatively simple amines in which the amino acid nitrogen is not in a heterocyclic ring. They are biosynthesized from aminoacids and are basic .

Examples are mescaline (6) and ephedrine.(7)



Mescaline

(6)

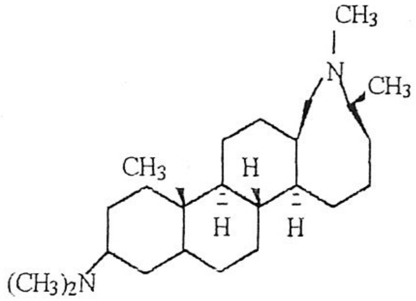


Ephedrine

(7)

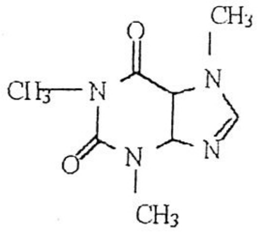
5-3: Pseudo Alkaloids:

The pseudo alkaloids are not derived from an aminoacid precursor . They are usually basic. There are two important series of alkaloids in this class. The steroidal alkaloids e.g.: conessine (8) and the purines e.g.: Caffeine (9).



Conessine

(8)



Caffeine

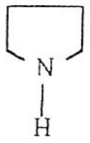
(9)

5-4: Some Typical Classes Of Alkaloids With Respect To Their Chemical Structure:

5-4-1: Pyrrolidine Alkaloids:

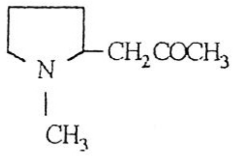
Pyrrolydine (10) is a minor alkaloid of tobacco, it is also found in wild carrot.

An other example of this group is Hygrine (11), Nicotine (12).



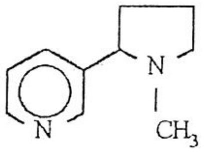
Pyrrolidine

(10)



Hygrine

(11)



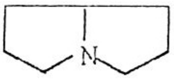
Nicotine

(12)

Nicotine (isolated in 1809 (dibasic) from tobacco)

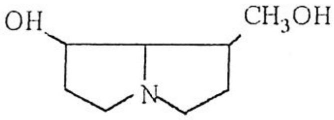
5-4-2: Pyrrolizidine Alkaloids (poisoning of cattle and horses) :

Two examples are shown below:



Pyrrolizidine skeleton

(13)



Platynecine

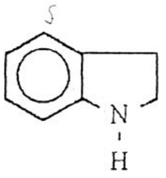
(14)

5-4-3: Indole Alkaloids:

There are about 1400 indole alkaloids [6] known up to now. They are derived from aminoacid tryptophan. It is the largest group of alkaloids. They are found especially in some plant families such as apocynaceae, rubiaceae and longaniaceae.

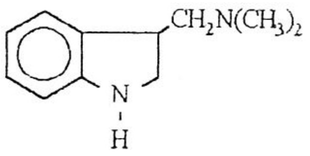
Some examples are as follows:

5-4-3-1: Simple Indole Alkaloids:



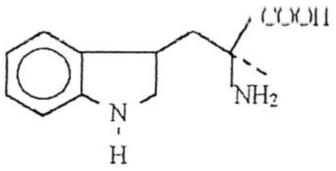
Indole Skeleton

(15)



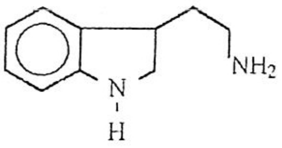
Gramine

(16)



Tryptophane

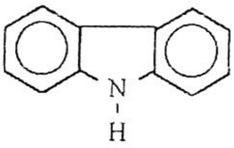
(17)



Tryptamine

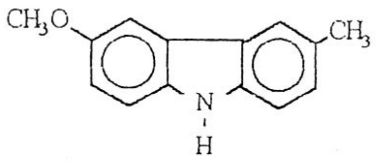
(18)

5-4-3-2: Carbazole Alkaloids:



Carbazole Nucleus

(19)

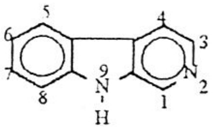


Glycosoline

(20)

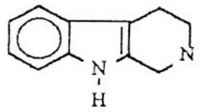
5-4-3-3: *β-Carboline (Harmala Alkaloids):*

The Harmala alkaloids are derivatives of the β-Carboline system



β- Carboline

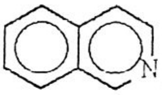
(21)



Tetrahydro-β-Carboline

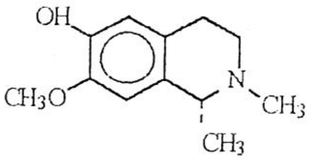
(22)

5-4-4: *Isoquinoline Alkaloids:*



Isoquinoline Nucleus

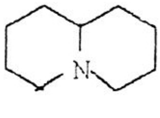
(23)



(+) Salsoline

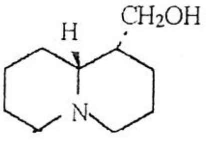
(24)

5-4-5: *Quinolizidine Alkaloids:*



Nucleus

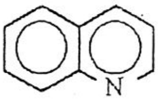
(25)



(-) Lupinine

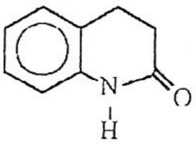
(26)

5-4-6: Quinoline Group:



Quinoline

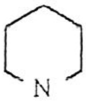
(27)



Quinolone

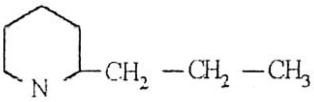
(28)

5-4-7: Piperidine Alkaloids:



Piperidine

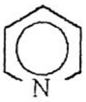
(29)



Coniine

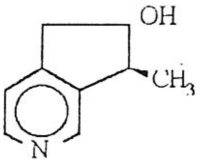
(30)

5-4-8: Pyridine Alkaloids:



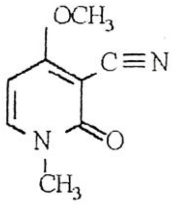
Pyridine

(31)



Cantleyine

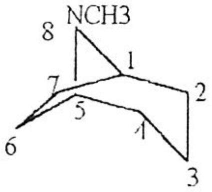
(32)



Ricinine

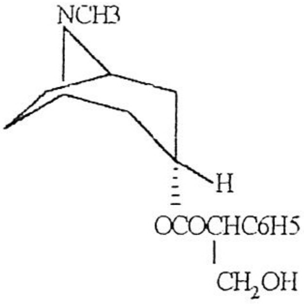
(33)

5-4-9: Tropane and Related Bases:



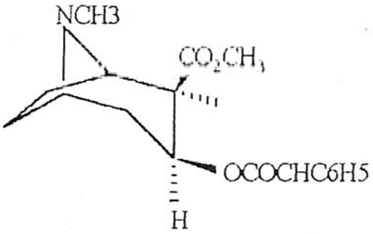
Tropane nucleus

(34)



Atropine

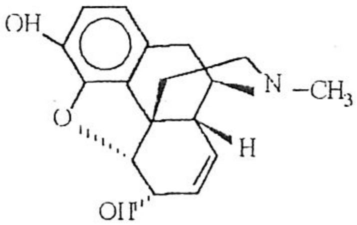
(35)



Cocaine

(36)

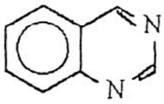
5-4-10: Morphine Alkaloids:



Morphine

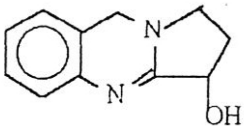
(5)

5-4-11: Quinasoline Alkaloids:



Quinasoline Nucleus

(37)



Vasinine (peganine)

found also in P.Harmala

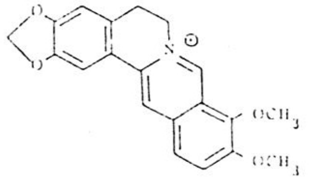
(38)

6- Physical and Chemical Properties:

6-1 : Physical Properties[٤,7]:

Most of the alkaloids that have been isolated are crystalline solids with a defined melting point or decomposition range.

A few alkaloids are amorphous gums, and some such as nicotine and Coniine are liquids. Most alkaloids are colorless, but some of the complex, highly aromatic species are colored. e.g. : Berberine(39) is yellow.



Berberine

(39)

The solubility of the alkaloids and their salts is of considerable significance in the pharmaceutical industry, both in extraction of the alkaloids from the plant or fungus, and in the formulation of the final pharmaceutical preparation. In general the free base of alkaloids is soluble only in an organic solvent. The salts of alkaloids and the quaternary alkaloids are normally highly water soluble.

6-2 : Chemical Properties:

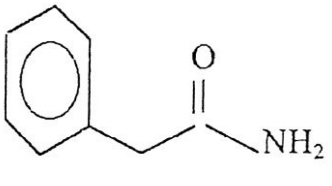
The most distinct chemical property of most alkaloids is that they are basic. This property is of course dependent on the availability of the lone pair of electrons on nitrogen. If the functional groups adjacent to nitrogen are electron releasing, for example an alkyl group, the availability of the electrons on nitrogen is increased and the compound is more basic.

e.g.: Triethylamine (40) is more basic than diethylamine(41) which in turn more basic than ethylamine(42)

$(C_2H_5)_3N$	$(C_2H_5)_2NH$	$(C_2H_5NH_2)$
(40)	(41)	(42)

Alternatively, if the adjacent functional group is electron withdrawing (e-g: a carbonyl group), the availability of the lone pair is decreased and the effect is to make the alkaloids neutral or even slightly acidic.

A typical example is the amide group of compounds.

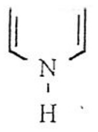


Phenylcetamide

(43)

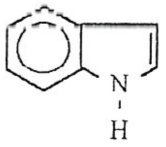
It will become evident that the alkaloids contain a diversity of nitrogen heterocyclic systems, and therefore it is pertinent to discuss the basicity of some of the most common heterocyclic systems found in alkaloids. The pyridine nucleus contains 6 Π electrons within the heterocyclic ring. The lone pair on nitrogen is therefore available and pyridine is basic. The carbon-nitrogen double bonds reduce this basicity somewhat however and pyridine (31) is less basic than its saturated analogue Piperidine (29).

Turning attention to the corresponding five-membred ring systems, pyrrole (44) is only fully aromatic (4 Π +2e) when the lone pair of electrons in nitrogen is involved in the aromaticity, hence pyrrole (44) and indole (15) are not basic.



Pyrrole

(44)



Indole

(15)

7: Detection, Isolation, Purification, and Structure Elucidation:

It is rare that alkaloids [2, 3, 8, 9] are found by chance, alkaloids chemists normally search for alkaloids quite deliberately. Indeed often one group of alkaloids will be sought to the exclusion of others. How is it possible to be able to evaluate a plant specifically for alkaloids, and how are these natural products isolated and characterized?

7-1: Detection:

Since alkaloids continue to provide new structures and interesting pharmacological activities, it has been necessary to develop simple methods for detection of alkaloids in plant materials.

Two methods are the most reliable to screen for the alkaloids containing plants. The first method consist of extracting 20 g of dried plant with an alcohol then, the residue after filtering is evaporated and taken up in water.

After acidifying, an appropriate precipitating reagent is used. If either test is positive, a confirmatory test is made in which the acid solution is basified, the alkaloids are extracted into an organic solvent, and then are back-extracted into aqueous acid. If the acid solution yields to a precipitate with the reagent, the plant contains alkaloids.

The second method is somewhat different since it relies on the conversion of alkaloids to their free bases by moistening the plant material with dilute aqueous ammonia. The alkaloids are then extracted with an organic solvent and next, removed as hydrochlorides by addition of hydrochloric acid. The filtered aqueous solution is screened for the presence of alkaloids with an appropriate reagent .

Mention has been made of several precipitating reagents used in screening for alkaloids. The reagents are often based on the ability of the alkaloids to combine with high atomic-weight metals such as Mercury , Bismuth, Tungsten, or Iodine. Thus Mayer's reagent undoubtedly the most popular, contains potassium iodide and mercuric chloride, and Dragendorff's reagent contains bismuth nitrate and potassium iodide in aqueous acid.

Unfortunately, several other types of compounds may also give precipitates with these heavy metal reagents. Examples are proteins, coumarins, α pyrones, hydroxy-flavones, and tannins. Such reactions are termed « false-positive ». For an attempted confirmation by the back-extraction technique precipitation is not usually successful. The preparation of these various reagents is summarized in the **Appendix.**

7-2: Isolation:

An important goal of alkaloids investigations is the isolation of the alkaloids present in the material to be investigated in their genuine form without any formation of artifacts. Much of the activity in the field of the alkaloidal investigations has been directed towards the development of optimum methods for the isolation of alkaloids and alkaloid mixtures with the avoidance of undesirable alteration to them.

As the alkaloids usually occur in plants as salts of organic plant acids and inorganic acids together with often complex mixtures of water-soluble compounds, such as gums proteins, mineral salts, tannins, lipids (fats and oils) and resins, it is often a great problem to remove all of these non alkaloidal compounds, during the isolation and purification of the alkaloids.

The extraction and isolation of alkaloids can be carried out in various ways depending on the nature of the alkaloids in question and the primary extraction is usually affected with organic water-immiscible solvents after the liberation of the alkaloidal bases from their salts by treatment with a mineral base, as prolonged contact with strong bases my lead to alterations to many alkaloids, such as hydrolysis of ester alkaloids, and as strong bases also cause the formation of soaps, if fats are present ammonia is most commonly used. Ammonia is sufficiently basic to liberate most of the common alkaloids without much risk of undesirable reactions. Also, as ammonia is volatile, it can be removed afterwards.

However, because of problems that may occur when the alkaloidal bases are liberated by treatment with alkali and extracted with organic solvent, such as alterations to the alkaloids under the influence of dichloromethane or chloroform, extractions of the lipids present in the material to be investigated should be performed with other water-immiscible organic solvents.

Extractions of the alkaloids as salts by means of water alcohol mixtures is often preferred. The risk of alteration to the genuine alkaloids present in the material is less under such conditions. Whereas extractions with most organic solvent such as benzene, chloroform and diethyl ether give extracts containing all kinds of lipids and resins, extraction made with water and water-alcohol mixtures will give extracts containing various polar compounds, such as proteins, gums and mineral salts.

7.3:The methods of extraction mentioned above are displayed in details as below:

7.3.1: Extraction with a non-polar solvent:

Plant materials [10] often contains quite substantial quantities of very non polar fats and waxes. Because these compounds frequently cause problems due to emulsions when they are subject to partition, they are often removed from the plant material as an initial step by percolation of the plant material with petroleum ether or alike.

After defatting, several procedural choices are available. The plant material may be extracted with water, with ethanol or methanol, with aqueous alcohol mixtures, or with acidified aqueous alcohol solutions. The alcohol solution is then evaporated to a thick syrup and the residue partitioned between an aqueous acid solution and an organic solvent. The aqueous phase is then basified with ammonia. The basic aqueous solution is next extracted with a suitable organic solvent. The alkaloid-containing solution is dried with an agent such as sodium sulfate, filtered and evaporated to afford the crude alkaloid residue. (figure 02 and 03).

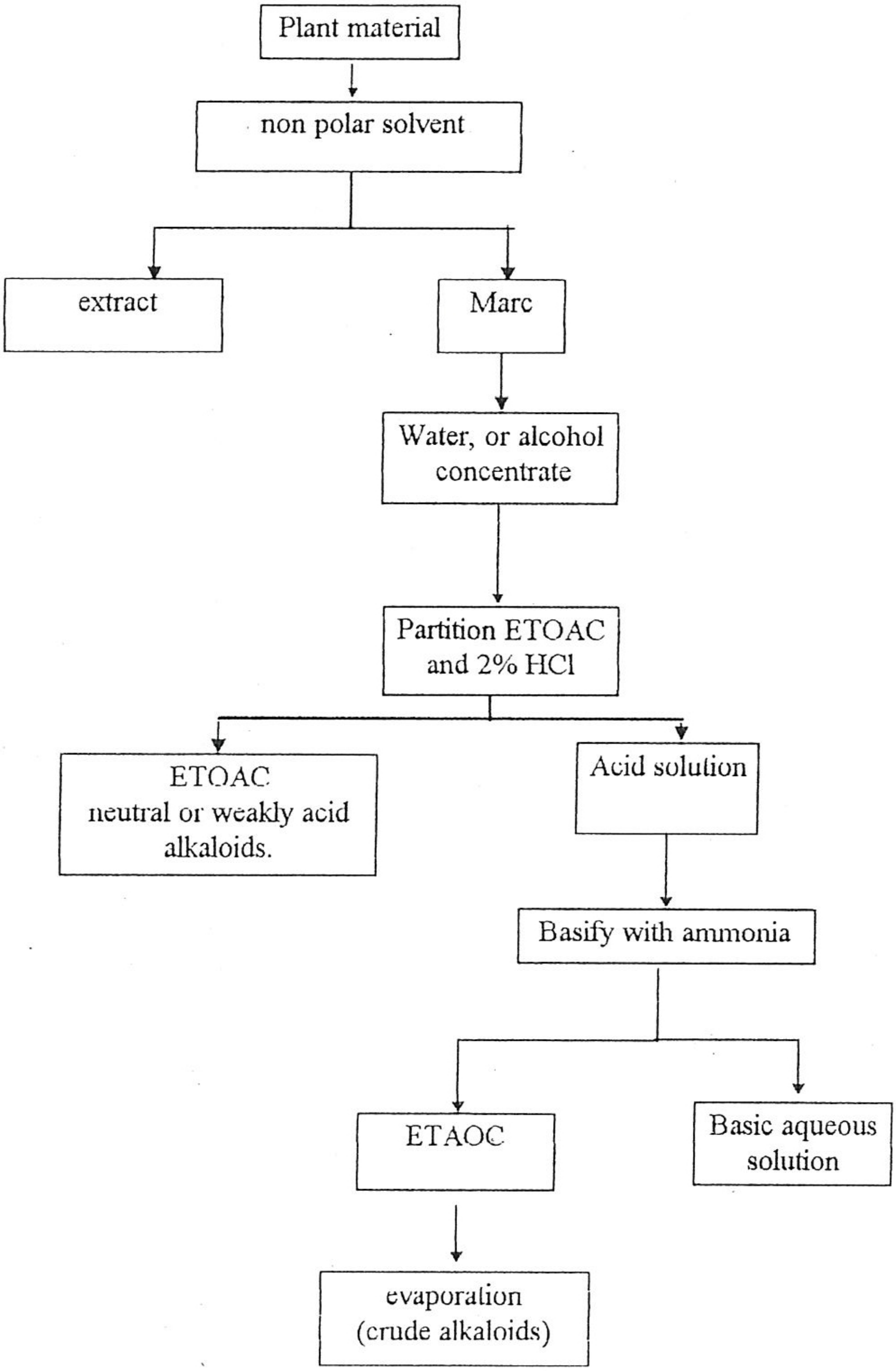
7-3-2: Extraction with a polar solvent:

The plant material [10] dried and powdered is subject to a polar solvent such as methanol, ethanol or isopropanol. The treatment with an alkali is not achieved in this step. Then, the alcohol solution is subject to further purification as shown in figure 04.

7-3-3: Extraction with an acidic solution:

Acidic solution [10] is an excellent solvent for alkaloidal extraction but it presents the disadvantage to remove many other undesirable substances such as proteins, sugars, resins and so on. .For the rest of the operation, similar steps as the other procedures are conducted.

Figure-2: Extraction with a non-polar solvent.



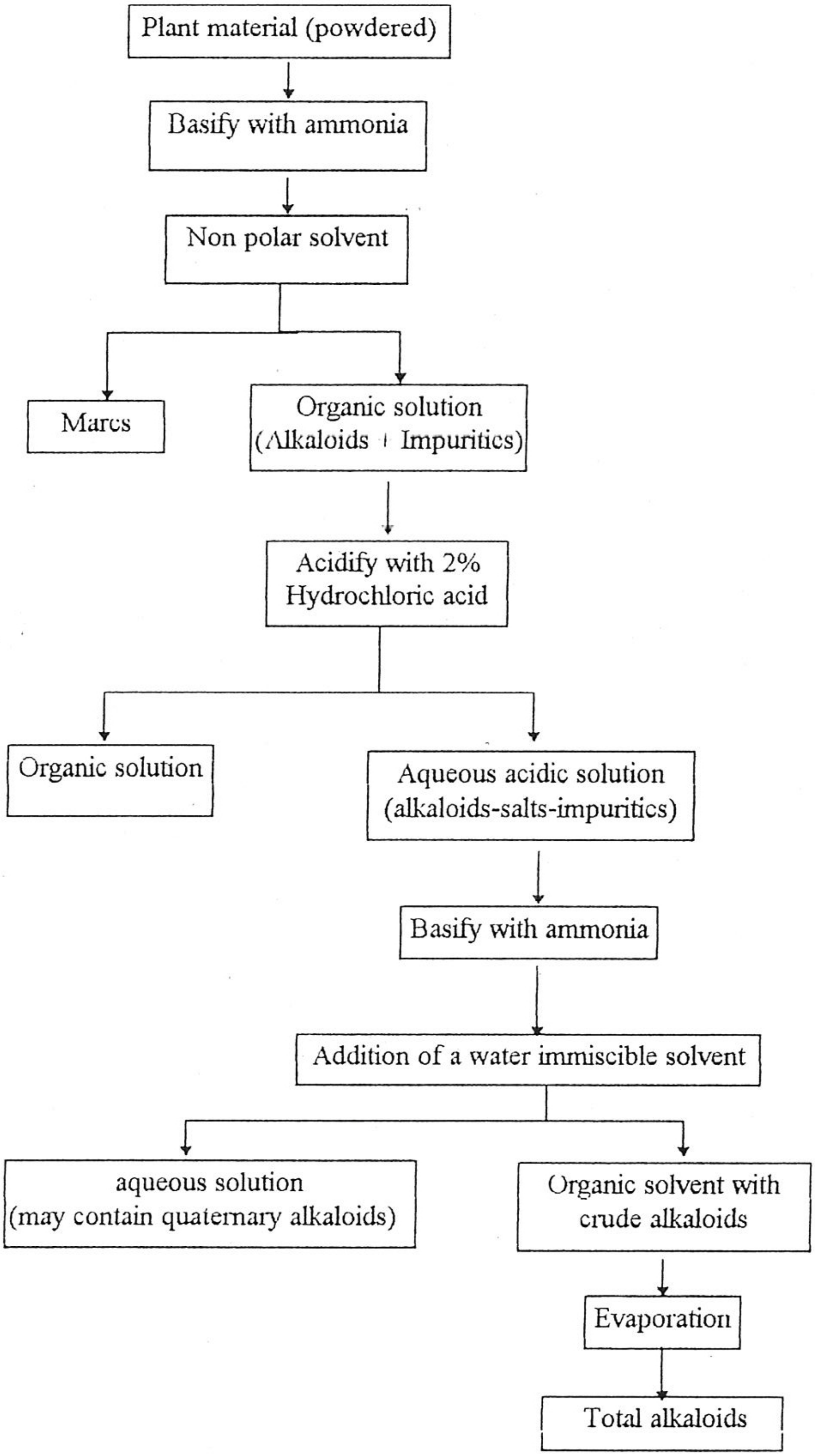
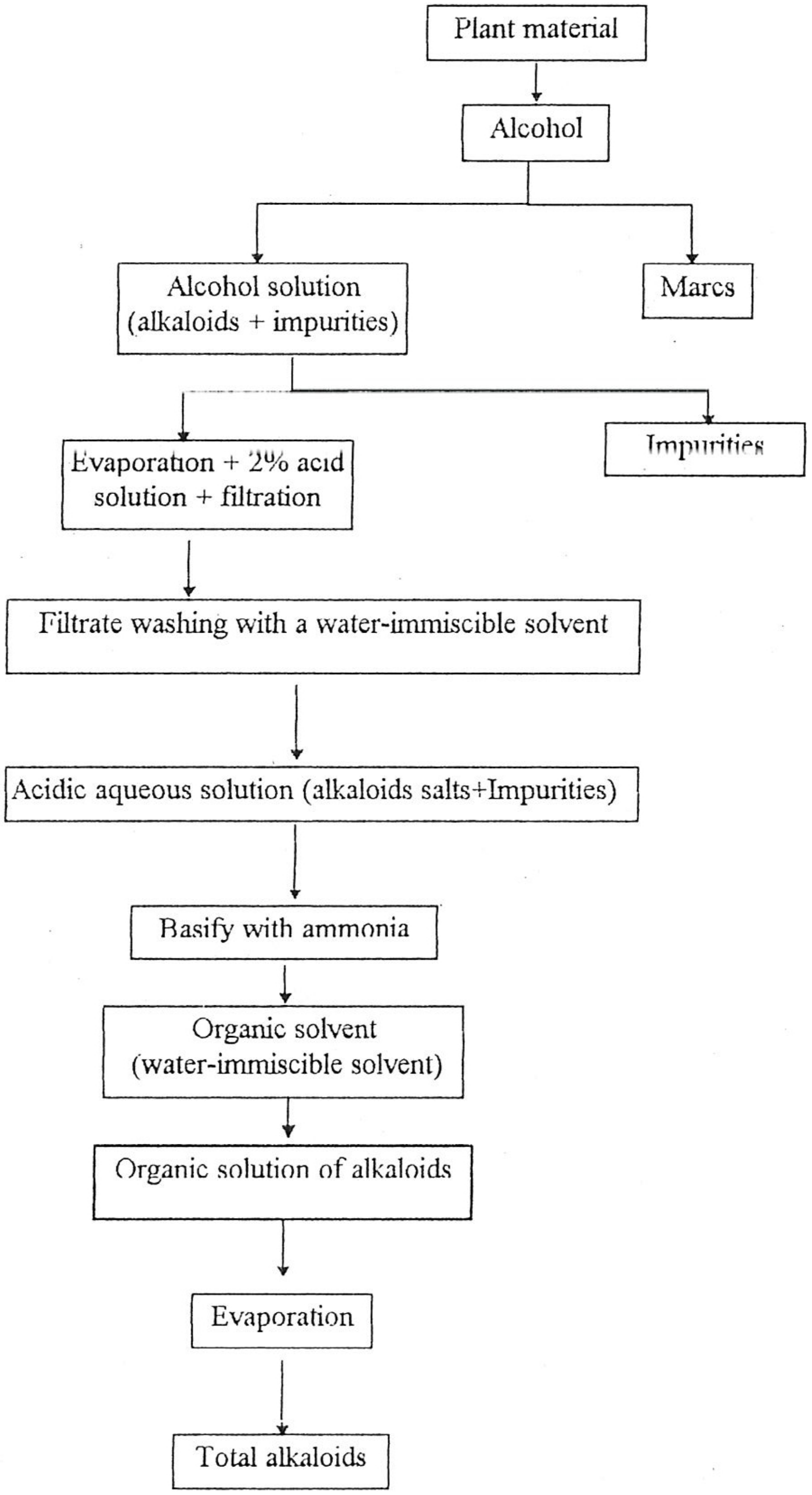


Figure-3: Extraction with a non-polar solvent after basifying

Figure-4: Extraction with a polar solvent:



7-4: Solvents and Artifact Formation:

During the extraction and analysis of alkaloids [11] it should be borne in mind that the stability of alkaloids varies widely, Some alkaloids are sensitive to light, others to pH and heat and some even to various organic solvents. Some organic solvents can influence the decomposition rate of sensitive alkaloids, but reactions can also occur between various solvents, or contaminants in solvents, and alkaloids. Because of the intensive use of organic solvents in the extraction, isolation and analysis of alkaloids, some of the possible interactions between organic solvents and alkaloids are discussed below. The commonly used organic solvents in alkaloid research belong to various chemical groups:

Aliphatic and aromatic hydrocarbons (benzene, toluene, cyclohexane).

Alcohols (ethanol, methanol).

Ethers (diethyl ether, methyl ethyl ether, dioxane, tetrahydrofuran).

Esters (ethyl acetate).

Ketones (acetone, methyl ethyl ketone).

halogen-containing compounds [12,13,14] (chloroform; dichloromethane)

Decomposition may take place in all types of solvents. Particularly in chloroform solutions photochemical decomposition may be accelerated. In alcoholic solutions alkaloids are usually more stable.

Ethers [15] are often contaminated with peroxides, which may cause oxidation of the alkaloids.

Ketones [16] are well known as artifact formers and they may react with alkaloids: e.g. Berberine (39). During column chromatography ketones may give condensation products with ammonia, forming compounds with alkaloidal characteristics [16]. Self condensation of acetone during liquid chromatography is also a problem that may be encountered with this solvent.

Halogen-containing solvents are widely used in alkaloid research, chloroform is in particular one of the most suitable solvents because of its relatively strong proton donor character,[17] however, the halogen-containing solvents are very active in terms of artifact formation. Even decomposition of alkaloids is accelerated in chloroform solution.

Dichloromethane reacts readily with tertiary nitrogen atoms, yielding quaternary alkaloids which are insoluble in organic solvents. With strychnine it was found that the tertiary alkaloid [18] was converted completely into the quaternary dichloromethyl compound within 48h.

Chloroform may be contaminated with other halogen-containing compounds (bromochloromethane and dichloromethane). Small amounts of dichloromethane and bromochloromethane may also cause the formation of quaternary chloromethyl compounds [19,20,21] of tertiary amines in chloroform solutions. 1,2-Dichloroethane also has alkylating properties.

Basic nitrogen-containing compounds as ammonia, diethylamine and Triethylamine are widely used in the analysis of alkaloids. Ammonia is often employed in connection with the extraction and purification of alkaloids. However, also with ammonia artifacts can be formed in some instances. Ammonia reacts with aldehyde functions, converting a two-nitrogen containing alkaloid to a three-nitrogen containing alkaloid [22].

The conclusions to be drawn from the foregoing are that solvents should be freshly distilled, ethers should be checked for the presence of peroxides, and great care should be taken when chloroform is used.

In a prolonged column chromatographic separation the use of chloroform may lead to artifact formation. Small amounts of bromochloromethane and dichloromethane and related contaminants in chloroform may lead to quaternization of tertiary alkaloids present.

Hydrocarbons are to be preferred because of the small risk of artifact formation.

7-5: Purification of the Alkaloids Extract:

With the crude, complex alkaloids extract in hand, the next step is separation into the individual components. There are a number of conventional methods available, and the choice of appropriate method or combination of methods will depend entirely on the particular alkaloid mixture.

7-5-1:Direct Crystallization:

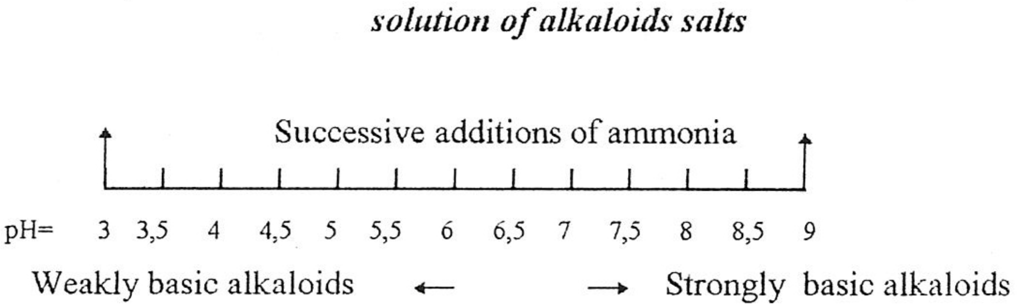
Eventhough this is potentially the simplest procedure, It is rarely successful for the isolation of a pure alkaloid [2] unless one alkaloid is present a very important constituent or if it is particularly insoluble.

7-5-2:Gradient pH Technique:

This technique [2] was devised for the isolation of catharanthus roseus alkaloids. It relies on the different basicities of alkaloids. The crude alkaloids mixture is dissolved in 2% tartaric acid solution and extracted with benzene or ethylacetate. The first fraction will contain the weakly basic alkaloids, the pH of the aqueous solution is then raised by 0,5 pH increments to pH 9 extracting at each pH with the organic solvent.

The changes in pH permit the gradual separation of weakly basic from medium basic and strongly basic alkaloids.

The above concept is represented as follows :



7-6 : Chromatography of alkaloids:

The great number of alkaloids isolated and characterized in the past years can be traced directly to the introduction of chromatography as a purification technique prior to carrying out any preparative-scale separation. It is normal to develop thin-layer chromatographic systems. Such separation systems may be on silica gel, alumina, cellulose powder or kieselghur.

In fact the application of the chromatographic techniques in the isolation, purification and fractionation of alkaloids has also created new possibilities in the important field of alkaloid research which started with the isolation of morphine (5) by in 1806.

The aim of TLC analyses is to determine the suitable solvents to be used subsequently so as to separate the alkaloids from the mixture effectively.

7-6-1: Uses of Thin-Layer Chromatography:

The perquisite for successful use of flat bed chromatography for analytical and micropreparative purposes is not only the correct choice of experimental conditions, but also that the substances chromatographed have suitable properties.

In order to chromatograph a given substance successfully, it must fulfill the following requirements: first it must be detectable on the chromatogram, further it must be soluble at least to the extent that it can be applied in the form of the solution on the start and can then move with the mobil phase; it must also be non-volatile, so that it will not evaporate after its application onto the start or during chromatography and drying of the chromatogram; It must be stable under the conditions of chromatography, i.e. resistant to the action of light, air, and the solvents used.

7-6-1-1: Use in qualitative analysis:

The commonest task [23] in this field is the test of purity and the analysis of a known mixture. This involves the determination of the number of substances investigated and requires a suitable choice of solvent system and detections reactions. A system should be found in which the suspected components have different R_f values. This is because sometimes such a large amount of the sample must be applied on the chromatogram, that the excess causes strong tailing of the main component.

7-6-1-2: Quantitative Analysis:

7-6-1-2-1 :Use in situ methods :

The methods of quantitative analysis [23] can be classified into those applied *in situ* and those applied after elution. The *in situ* methods are based on a direct quantitative evaluation of the spots on the chromatogram, either by visual (subjective) inspection or by using the appropriate instrumental technique (objective evaluation). Use is made of the relation between the intensity, area or length of the spot and the analytical concentration. In all instances it is important to find a suitable concentration range within which the corresponding technique is most sensitive for a given case. The shape of the spot also plays its role: very often spots of higher R_f values, which are well developed in consequence of diffusion in all directions, are better evaluated than the spots near the start or those washed into the solvent front. Small amounts of impurities are an exception. Therefore, it is better to apply the same volumes of solutions of various concentrations onto the start (this has another advantage: The exact volume need not be known than to apply different volumes of the same concentration).

In cases when a relationship is found between the area of the spot and the analytical concentration of the substance determined, it is often sufficient the copy or to photograph the spots and then either to submit them to planimetry or to cut them out and weigh them.

7-6-1-2-2: Methods After Elution :

Chromatography serves only as a separation method and the separated substances are isolated from the chromatogram and then determined by a suitable analytical method. Usually the amount applied is larger than in the in situ methods and therefore the sample should be applied in form of bands rather then drops.

7-7: Structure elucidation:[24]

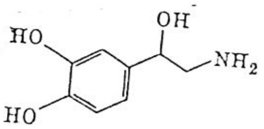
Although a number of alkaloids are described in the nineteenth and early the twentieth centuries, their structure took many years to be deduced or were left unsolved. This was the drawback of the classical organic chemistry when structures were obtained by degradation and the demonstration that certain functional groups were present in the degradation products.

At present, if the isolated alkaloid is known, it may take only minutes to prove its identity. If the alkaloid is new, a converted effort may result in a tentative structure within days and be proven within a week. Particularly difficult structures may take up to 6 months, but these instances are becoming increasingly rare.

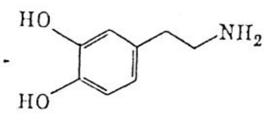
The reasons for this dramatic change in structure elucidation have been the introduction of physical techniques developed to determine and define both major and minor structural fragments, The days of degradation to characterize every individual carbon atom are over.

8: Pharmacology- The basic concepts:

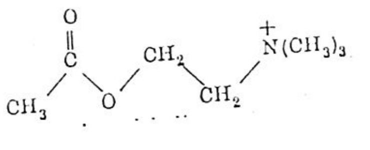
The most principal actions of alkaloids [2] in human body is on the nervous system. The nervous system perceived environmental changes rapidly to a specific processing center. The fundamental unit involved in this process is the neuron, with the information being transferred across a minute gap (2 × 10^{-5} mm) called synapse. Although within a neuron information is passed electrically, between neuron information is passed chemically by compounds known as neurotransmitters. The four neurotransmitters generally recognized are acetyl-choline (45), noradrenaline (46) dopamine (47), and serotonin (48). The reactive site for these chemicals on the adjacent neuron is called a receptor .



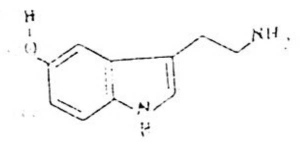
noradrenaline(46)



dopamine(47).



acetyl-choline(45),

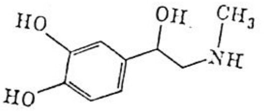


serotonin(48).

A drug may mimic, facilitate or antagonize a normal process, It may also increase, decrease or disrupt The normal activity of the cell. The formation of some of the neurotransmitters mentioned above (choline) requires sodium ions, glucose and oxygen. Blocking this synthesis causes transmission to fail.

Other drugs occupy the receptor side and prevent the neurotransmitters from acting or elicit responses that resemble stimulation (of neurotransmitters i.e. = release noradrenaline). This has become the basic for using the correct drug for a particular disease state.

Epinephrine (49) and ephedrine (7) are good examples.



Epinephrine

(49)

Other Examples:[26]

medicines:	Ephedrine (7) respiratory ailments.
	Ricinine (33)castoral,purgative.
poisons:	Coniine(30) (hemlock).
narcotics:	morphine(5) (opium).
	cocaine (36) (stimulant south America Indians).
Stimulant:	caffeine (9).

II :PLANT STUDY

1: Description:

Overspread ion the Mediterranean [26-30] coast stretched eastwards up to Iran, and over, this plant overgrows the pasture-lands as well as the fallow lands, ruins and road borders. It has green starry clumps of white flowers, and releases an unpleasant smell. It grows on unspoiled lands, grazing lands, sandy soils, very common on high lands and in northern Sahara.

It is an overlasting branched plant, with leafy stems, up to 80cm high, alternate leaves with deep and numerous linear divisions ending in a point.

Lonesome flowers at the summit of each tug, passed beyond by the 5 sepals of linear divisions, corolla of about 3cm in diameter of 5 oval pedals, white inside, greenish outside, greenish ovaries and capsuled fruit.

Flowering period: Spring (May , June)

Fruit period: Summer (June , July).

This plant grows widely in Algerian eastern region especially near Ain M'lila where a village is called after its name « El-Harmlia ». It is used in form of compress mainly to diminish rheumatism pain around the aching part of the body. It is as well very famous for its ability to heal intestinal diseases and kill parasites.

2: Taxonomic classification : [27-30]

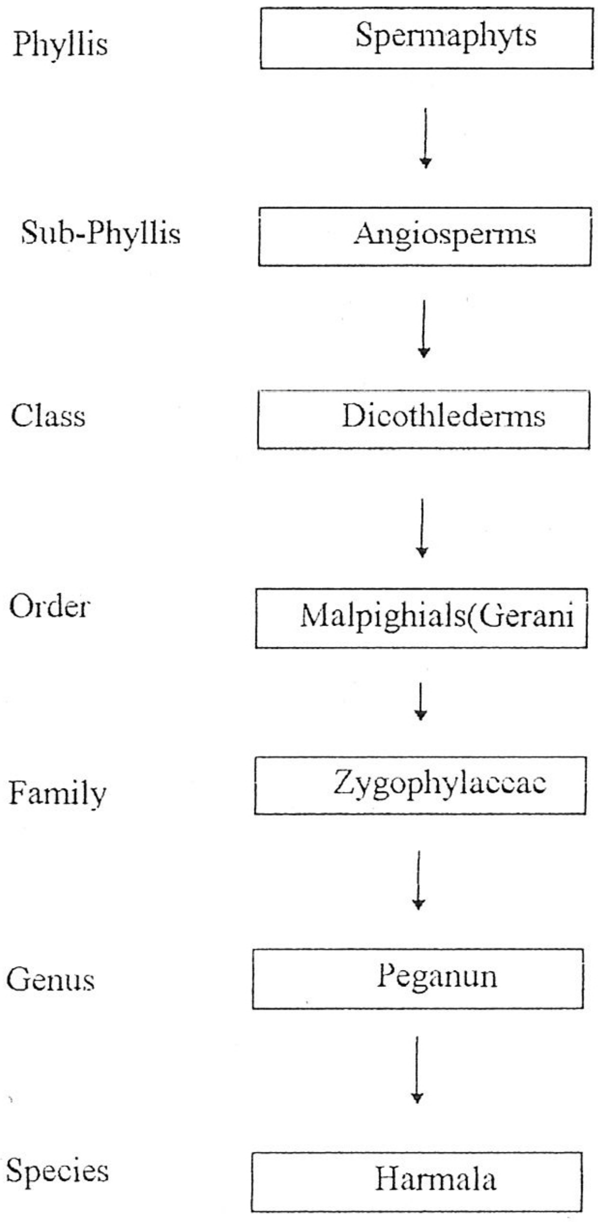
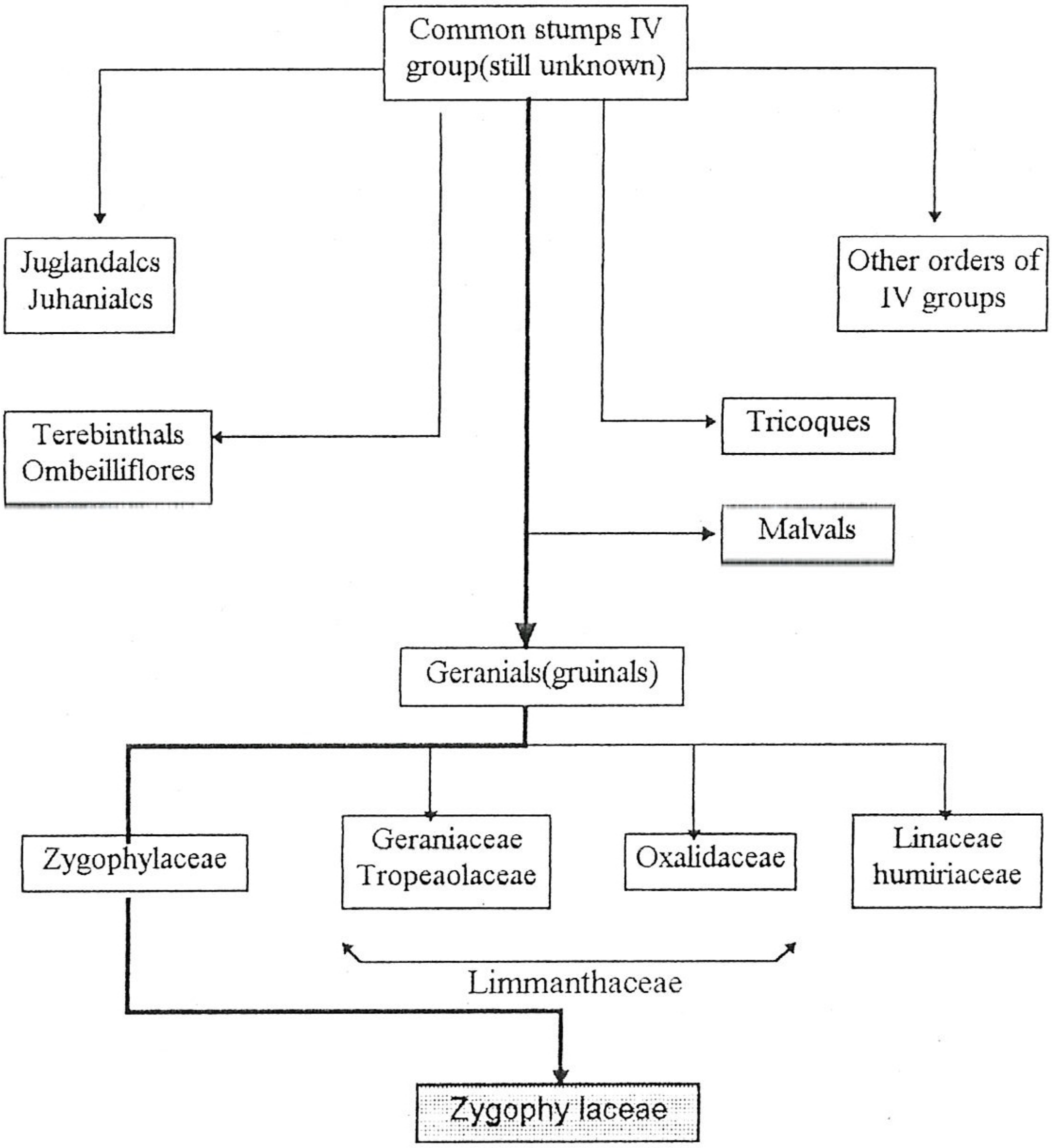


Figure :5

3: Systimatic of « Peganun.h » in the plant kingdom :[28-31]



Tribulusl	*Zygophyllum L*	*Flavonia.L*	*Seetzenia*	*Peganun.L*	*Nitravial*
A-T:Congipetalus 1- SSP alatus 2-SSPmacropterus 3-Terrester L	Z. Simplex -Z. Cornutum -Z. Album SSP Geslini SSP Abum	F- Olivieri F- Arabical F- Cretical.L F-Flamandibatt	S-Africana	Harmel	N.Retusa N.Shoberil

Peganun Harmala

Figure :6

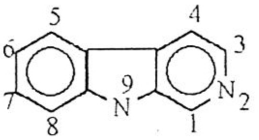
4: Harmala Alkaloids:

The harmala alkaloids [32] are derivatives of the β-Carboline system numbered as shown (19). This system particularly in its 1, 2, 3, 4 Tetrahydro form, occurs requently in the indole alkaloid series and there are literally hundreds of monoterpenoid derived indole alkaloids containing this unit as part of their skeleton.

In β-Carboline (19) itself nitrogen is weakly basic , but in the Tetrahydro derivative (20) the pepiridine nitrogen is now quite strongly basic. The indole NH is of course acidic in both cases.

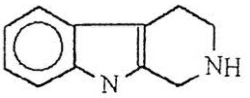
Most of the Harmala alkaloids are substituted at C-1 by a methyl groups, and the parent compound having this structure is known as harman.

These alkaloids have been found in several families, including the leguminosae, but most famously are associated with the seeds of Peganun Harmala.



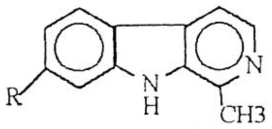
β-Carboline

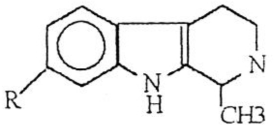
(19)



Tetrahydro-β-Carboline

(20)





Harman (50)	→	R = H	R = H (53)	→	Harmalan
Harmine (51)	→	R = OCH_3	R= OCH_3 (54)	→	Harmaline
Harmol (52)	→	R = OH	R = OH (55)	→	Harmalol

5: Back ground work:

In 1960: A-Schipper and O.H Volk [33] had isolated four Harman alkaloids and one Quinazoline alkaloids from Peganun Harmala in varying amounts depending on the time of year and the part of plant extracted. Herder reagent $[HgI_4]^{-2}$ was used in the essay for total alkaloids.

Paper Chromatography of the extractions from various parts of the plant were run by using Bu.OH saturated with 0,1 NH_4Cl.

Standard R_F values were Harmine (0,68), Harmaline(0,55), Vasinine (Peganine) (0,38) Harmalol (0,26). All alkaloids are presented (3,8 → 5,8%) of dry weight in an approximate ratio Harmine, Harmaline, Vasinine + Harmalol) of 61, 29, 10.

In 1960: Sallumazzaman siddiqui [34] isolated a new base Harmidine

(I) $C_{13}H_{14}ON_2$ from Peganun Harmala in 1,7% yield m.p 257-8° from dilute alkaline solution with a proper reagent. Reaction of this with Br_2 in $CHCl_3$ at 0° gave $C_{13}H_4ON_2Br_2$.

Reaction of this with aq NH_4OH gave monobromoharmidine, refluxing (I) with aqueous « H I» gave Harmidol $C_{12}H_{12}ON_2$.

In 1963: D.Groeger H.Simon [35] extracted Harman alkaloids with 3:1 $CHCl_3$-isoprOH and separated by paper chromatography.The paper was impregnated with pH = 7,2 Mackelvaine buffer and the mobile phase was BuOH saturated with H_2O .

(the alkaloids were developed with (10:1,5:5) BuOH . ACOH . H_2O).

In 1964 : Salimuzzaman Siddiki and Rashid Kamel isolated [36] three alkaloids from the seeds of Peganun Harmala. In addition to Harmine (I), Harmidine (II), a new crystalline constituent pegaline (III). The pH of an aqueous solution of the dark red residue from the ETOH extract of 250g of ground seeds was increased to 6,5 with dilute NH_4OH. Addition of $(NH_4)_2SO_4$ gave a dark brown spongy precipitate which was extracted with 2% acetic acid. Addition of excess KI to a solution of this extraction plus the main solution gave a sparingly solution salt which after washing with dilute KI gave golden yellow crystals from MeOH treatment with 10% NH_4OH. Solution in dilute HOAC and elevation of pH to 7,4 with dilute NH_4OH gave a nearly colorless solution. Other fractions were collected in like manner but at pH = 8 and above. Recrystalization of the two fractions from MeOH -C_6H_6 (1:1) gave a 1,11% of colorless (I) 2nd fraction gave (II). The reddish brown crystals, which appeared when an alkaloid extract of 20g of seeds was allowed to stand for 1 to 2 weeks and then concentrated give a greenish solution after washing with Petroleum ether. When concentrated, this yielded (I). Hcl, the tail fractions of this crystallization gave (III) (mp ≅ 295°).

In 1965: N.V Plekhanova and S.I. alktanova studied [37] the total content for alkaloids. Peganine (I), Harmine (II), Harmaline (III) odeoxypeganine (IV) and Oxopeganine (V) maximum content was found in the fruit period in upper parts. (3,01%) total 60 % of (I), In seeds (3,92 %) total 94,5 of (III) (IV) and (V) were the degradation products of (I).

In 1967: Claudio Naranjo studied [38] the psychtropic properties of Harmala alkaloids such as: Harmaline tetra hydroharmine, racemic tetra hydroharmine, 6 - 1 alkaloids were observed in human volunteers. There is a relation between Harmala alkaloids and substances found in animals such as adrenoglomerulotropine (I), a Hormone of the pineal body. (I) is identical to B-Methoxy Tetrahydro-Harman (II) which is formed in vivo from 5-Methoxy tryptamine and MeCHO. (II) is an isomer of tetra hydro-harmine, one of the alkaloids isolated from Banisteriopsis another substance 6-methoxyharmalan is derived in vivo from melatonin which in turns results from the methylation of acetylserotonin and enzyme which makes this methylation possible is hydroxyindole O-Methyl .

In 1977: (Aarons, Darryl H, Rossie G Victor, Orzechrouski, Rezmond F) reported[39] that Harmine-HCl, Harmaline HCl, Harmalol HCl decreased heart rate and increased pulse pressure peak aortic flow and myocardical contractile force in anesthetized dogs: (I) reduced systemic arterial blood pressure and total peripheral vascular resistance, Harmaline evoked decreases were frequently followed by a secondary increase, and the effects of Harmalol on these two parameters were inconsistent. A direct neg chromotropic effect of harmala alkaloids was suggested by observations of bradycardia in the isolated perfused rat heart and in the intact dog, neither vagotomy nor atropinization affected Harmala-alkaloids induced brady cardia in the dog. Reduction in femoral vascular resistance by the alkaloids was not appenently due to activation of cholinergic, β-adrenergic, or Histaminic (H_1) receptors.

6: Pharmaceutical Effects of Harmala Alkaloids :

Extracts of harmala alkaloids were notorious[40] for their psychotomimetic activity in man . This type of activity was probably due to the presence of Harmaline (54) which: in *vivo* dose of 1 mg/1kg and oral of 4 mg/kg produces psychotomimetic activity in man[41].

Harmaline (54) is used at one time in therapeutics against tremors of Parkinson's syndrome . The study of central effects of harmala alkaloids, conducted in order to understand their mechanism and E.E.G effects, revealed that Harmine (51) and Harmaline (54) caused excitation, tremors and ataxia in rats and cats (after implantation) of electrodes in various cortical and subcortical areas. Harman (50) produces at low doses a motor depression with a catatonic components. Higher doses led to clonic convulsions without any tremors[42]. High intravenous doses of Harmine (51) cause some hallucinogenic effects in mental patients[43] .

Harmaline (54) is about twice as potent of hallucinogen and more potent than mescaline (7) [38]. If injected intravenously; it produces almost instantaneous hallucination.

All Harmala alkaloids are ineffective as contact insecticides but active in vapor form, They are effective against algae in higher concentration. then were lethal to many water animals (frog, dauphin, mosquito), they were also lethal to molds, bacteria, and intestinal parasites.

III: EXPERIMENTAL

1: Preliminary Phytochemical Screening of The Different Organs:

The powdered material of each part of the Plant, that is, roots, stems, leaves, flowers, and seeds is collected at different periods of the year and subject to the following tests.

1-1: Steam Distillation [44] :

50 g of powdered material of each part of the plant is put in shaking flask then, 250-300 ml of distilled water is added and the distillate is extracted with ether. Subsequently the ether solution is filtered through Na_2So_4 non hydrate and the ether is evaporated at the lowest possible temperature .The remaining part is scanned and yields to appearance of a yellow layer at the water surface. Hence, presence of volatile oils.

1-2: Test for Glycosides and Carbohydrates [45] :

Plants and green algae absorb carbon dioxide and convert it into carbohydrate molecules such as glucose, sucrose, ... etc.

10 g of powdered material is mixed with 50ml of 2% tartaric acid in ethanol, then the mixture is heated over a water path, washed with ethanol over a filter paper. And the filtrate is then evaporated, dissolved in warm water and subject to the following tests.

5 ml of each extraction is put in a test tube to which 0,5 ml of alcohol solution of 15% alfa-naphtol is added. To the wall of the test tube concentrated sulfuric acid is added with maximum care so that a ring is formed at the bottom of the tube. The violet ring that separates the two phases indicates the presence of glycosides or carbohydrates.

1-3: Test for Ketoses [46] :

2 ml of each extract is mixed with 2 ml of Ammonium molybdate solution in a test tube. Just after, 1ml of acetic acid is added. The solution is heated over a water-bath, the blue color observed for a moment indicates the presence of ketoses.

1-4: Test for Cardenolides:

Hydrolysis of steroid glycosides gives Cardenolides.

1-4-1: Tadros' Test [47] :

1 g of powdered plant material of each part is percolated with 20 ml of distilled water, then filtered. 10 ml of filtrate is mixed with 10 ml of ($CHCl_3$, and ethanol). The organic phase is evaporated till dryness, and the precipitate is dissolved in 3 ml of acetic acid, simultaneously then displaced to a test tube .Some drops of $FeCl_3$ are added, and followed by adding 1ml of concentrated sulfuric acid on the tube walls with maximum care. The non-apparition of green blue color in the acidic phase indicates the absence of Cardenolides.

1-4-2: Belget's reaction [48] :

1ml of Belget's agent (9,5 ml of picric acid mixed with 0,5 of NAOH in ethanol) is added to 1 ml the neat alcoholic extract. The non-appearance of orange color reveals the absence of Cardenolides.

1-4-3: Antimony Trichlorid Test [46] :

10 g of each part of the plant material is extracted with 70% ethylic alcohol till the total drain out. The alcoholic extract is evaporated under low pressure over a water bath. A small amount of precipitate is warmed with a bit of antimony Trichlorid reagent. The non-appearance of a dark violet color leads to the absence of Cardenolides.

1-5: Test for Flavonoids [49] :

They are the most numerous class of naturally occurring oxygen heterocyclic compounds. They constitute an important group of yellow natural pigments.

10 g of each powdered plant part is percolated with 150 ml in HCl 1% solution during a whole night, then filtered. The filtrate is next subject to the following tests.

1- 10ml of above is made alkali with NH_4OH, the appearance of light yellow color on the upper parts indicates the presence of Flavonoids.

2- 5 ml of the filtrate is stirred with amyl alcohol. The alcoholic layer colored in yellow leads to the presence of Flavonoids

3- The aqueous solution of (2) is boiled with 3 ml of hydrochloric acid during 2 mn and cooled, the solution is than splited into 2 parts.

Part 1: is shacked with amyl alcohol, the appearance of the yellow color indicates the presence of glycosides flavons.

part 2: is subject to a piece of Mg. The appearance of the red color indicates the presence of glycosides flavons.

1-6: Test for Steroids [46] :

They are very important natural products since they have a diversity of biological effects, they lie at the core of one of the thorniest modern debating themes - Fertility control.

5 g of powdered plant material is extracted with 70% of Et. OH, the alcohol extract is evaporated and the precipitate is dissolved in $CHCl_3$ and filtered once more.

The filtrate is then divided into two parts :

Part1: To the $CHCl_3$ solution 1ml of acetic solution is added and followed by 1ml of concentrated H_2SO_4 on the walls of the tube layers and the solution gives no green color that proves the presence of unsaturated steroids and.

Part2: [50] To the $CHCl_3$ solution is added the same volume of H_2SO_4 on the walls of the tube. The Yellow color is not transformed into red color, thus absence of steroids derivatives.

1-7: Test for Saponins [51] :

They are sterol glycosides that have complex sugar components attached to the 3 hydroxy group. The molecules therefore contain a hydrophilic and a hydrophobic part and are surface active (detergents), hence the name Saponins, probably because they are hydrolyzed in the stomach.

2 g of powdered material is boiled which 80 ml of distilled water, filtered and cooled, and the filtrate is well Shaken, the appearance of a foam which lasts a bit of time reveals the presence of Saponins.

1-8: Test for Tannins [52] :

They are complex mixtures of compounds, they appear to be secondary metabolites. They occur in high concentration in plant tissues and might be of use to the plant against parasites.

10 g of the powdered plant is extracted with 50% water alcohol (Et.OH) mixture. The filtrate is screened for tannins as follows .

1: Ferric Chloride Test:

Some ml of ET-OH solution is taken with some droplets of ferric chloride solution . The appearance of the green color indicates the presence of tannins.

2: Iron Complex Test :

5 ml of alcoholic extract to which is added 5 ml of Iron Ammonium Citrate and 1g of powdered sodium acetate, is boiled twice after being cooled. The appearance of a black and violet precipitate leads to the presence of the tannins.

1-9: Test for Alkaloids and Nitrogenous Bases [53] :

10 g of powdered material of each part of the plant is extracted with dilute hydrochloric acid. Each filtrate is made alkaline with ammonia and again extracted with $CHCl_3$ 3 times, each time with 20 ml.

The $CHCl_3$ is evaporated and the precipitate is dissolved in 2 ml of hydrochloric acid to be subject to Mayer, Wagner reagents. Furthermore, one droplet can be taken on a filter paper and sprayed with Dragendorff's reagent to make the above result more consistent.

Mayer's reagent gives a white precipitate, whereas Dragendorff's spray yields an orange color of the spots. Therefore presence of alkaloids.

The results are summarized in table1

Table 1

The active principal	**Roots**	**Stems**	**leaves**	**flowers**	**seeds**
Steam distillation (volatile oils)	+	+	+	+	+
Glycosides	+	+	+	+	+
Ketoses	+	+	+	+	+
Cardinolides	-	-	-	-	-
Flavonoids	-	+	+	+	+
Steroids/triterpenes	-	-	-	-	-
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Alkaloids	+	+	+	+	+

2: Extraction of plant material:

Prior to carrying out the extractive procedures ; the plant material of different parts is collected throughout the year .

The plant parts being collected are :
1. Roots
2. Stems
3. Leaves
4. Flowers
5. Fruits
6. Seeds

The plant material is then dried, powdered and subject to the following tests:

10 g of each part are taken and extracted for alkaloids with different solvent systems as below:

1- Petrol 2- Ether

3- Chloroform 4- Ethanol.

2-1: Extraction with Petrol :

100 g of powdered material

↓

Basify with ammonia

↓

extracted with petrol

↓

Addition of an acidic solution (1/10 HCl)

↓

Basify with ammonia (aqueous solution)

↓

Extraction with petrol

↓

Evaporation (after washing with NaCl)

↓

Crude alkaloids.

The results are listed in table 2 (in grams)

Table 2

Roots	Stems	Leaves	Flowers	Fruits	Seeds
1.09	1.98	1.82	0.98	2.21	2.64

2-2: Extraction with ether:

100 g of powdered material
↓
Basify with ammonia
↓
extracted with ether
↓
Partition with a dilute acidic solution.
↓
Basify (acidic solution) with 25% ammonia
↓
Re-Extraction with ether
↓
Washing with NaCl
↓
Evaporation (after washing with NaCl)
↓
Crude alkaloids mixture.

The results are summarized in table 3 (in grams)

Table 3

Roots	Stems	Leaves	Flowers	Fruits	Seeds
1.21	3.63	3.25	1.12	3.91	4.21

2-3: Extraction with chloroform:

100 g of powdered material of each part is subjected to an extraction procedure with chloroform- similar to the above extraction (with ether)

The results are shown in table 4

Table 4

Roots	Stems	Leaves	Flowers	Fruits	Seeds
1.11	3.22	3.02	1.09	3.71	3.89

2-4: Extraction with alcohol + water (1:1) :

100 g of powdered material

↓

Extraction with alcohol mixture (ethanol + water)

↓

Evaporation of the extract

↓

Partition between acidic solution (2% HCl) and ethyl acetate.

↓

Aqueous solution is basified with ammonia

↓

water immissible solvent (ethyl acetate)

↓

Organic solution of alkaloids

↓

Evaporation (after washing)

↓

Total alkaloids

The results are summarized in table 5

Table 5

Roots	Stems	Leaves	Flowers	Fruits	Seeds
1.22	3.12	2.92	1.01	3.83	4.05

The final results are listed in table 6

Table6

Plant material **solvents**	**Roots**	**Stems**	**Leaves**	**Flowers**	**Fruits**	**Seeds**
Petrol	1.09	1.98	1.82	0.98	2.21	2.64
Ether	1.21	3.63	3.25	1.12	3.91	4.21
Chloroform	1.11	3.22	3.02	1.09	3.71	3.89
Ethanol + water (1:1)	1.22	3.12	2.92	1.01	3.83	4.05

According to the above results we can infer that the suitable method to extract alkaloids is with ether and the maximum content is in the seeds. Consequently we opt for this method in the preparative scale extraction .

A further study is carried out on stems and leaves taking into account the periods of collection: e.i. :

Stems before flowering period	(May 95)
Stems during flowering period	(May, June 95)
Stems during the fruit period	(June, July 95)
Leaves before flowering period	(May 95)
Leaves during flowering period	(May, June 95)
Leaves during fruit period	(June, July 95)

the results are listed down in table 7

Table 7

S.B.Fl.	S.D. Fl.	S.D.Fr	L.B.Fl.	L.D.Fl.	L.D.Fr
3.12	3.22	3.68	2.88	3.15	3.25

N.B: The extraction is performed with ether.

2-5: Estimation of Alkaloids Content in Different Parts of the Plant at Different Periods [51] :

10 g of powdered material of each part of the plant at different growing periods is put into a percolator with a cotton plug at the bottom and at the top, then a sufficient volume of the ethanol 70 % is added and the sample is left for 24 hours before the solvent is allowed to flow down in a rate of 30 drops per minute and the operation is continued in the same way until all the alkaloids are run out (this could be made sure by negative Mayer reagent of the last drops). Next the alcohol extract is evaporated until the fifth and 10 ml of 0,1 N HCl is added with a continuous shaking to ensure the total dissolution of alkaloids. The acidic solution is then filtered through a bit of cotton in a separating funnel and the cotton is washed three times with 5 ml of 0,1N HCl to ensure the total removal of alkaloids. The acidic solution is extracted with 10 ml of $CHCl_3$ twice. The chloroform solution is again washed twice with 5 ml of 0,1N HCl. This later is made alkaline by adding ammonia. Then the alkaloids are extracted with 20 ml of $CHCl_3$ each time until the total removal of alkaloids. The $CHCl_3$ solution is washed, dried through non hydrated Na_2SO_4 and evaporated. The residue is then dissolved in 1 ml of $CHCl_3$ an 20 ml of 1/50 N HCl the solution is warmed until the evaporation. The titration is done with 1/50 N NaOH by using red of methyl as an indicator.

% alkaloids = [(Volume of acid - volume of base consumed) ÷ total mass of material] × N/50 × molecular weight of Harmine.

The result is : % alkaloids = 4.53 %

3: Thin layer chromatographic analysis of the different extracts:

The successive extracts are subjected to several solvent systems so as to be aware of the alkaloid contents as well as the best solvent system to be used subsequently for the separation by column chromatography.

Twenty solvent mixtures have been tried as shown below:

1- Chloroform-ethanol (95:5)

2- Ethyl acetate-Methanol (4:1)

3- Chloroform-MeOH (4 :1)

4- $CHCl_3$ -Acetone (1 :1)

5- Diethylether-ethyle acetate (1:1)

6- Ethyl acetate-isopropanol + ammonia (100:2:1)

7- Ethyl acetate-isopropanol + ammonia (80:15:5)

8- Ethyl acetate-isopropanol + ammonia (60:35:5)

9- Acetone- ethanol (85: 5)

10- Chloroform ethanol water (3: 1: 1)

11- Isopropanol-acetic-acid- water (10:10:1)

12- Benzene-ethylacetate / ethanol (2:2:1)

13- Chloroform / ethanol / ethylacetate / acetone (6:2:1:1)

14- Ethylacetate - Chloroform - Methanol (2:2:1)

15- Cyclohexane diethylamine (8:1)

16- Chloroform - isopropanol (4:1)

17- Methyl ethyl Ketone

18- n-Butanol

19- Benzene - diethylether - MeoH (6:4:1)

20- Me acetate - Isopropanol - 25% NH_4OH (45:35:25)

__The results are listed in the following table 8__

SS	ROOTS		STEMS		LEAVES		FLOWERS		FRUITS		SEEDS	
	Ns	Rf	Ns	Rf	Ns	Rf	Ns	Rf	Ns	Rf	Ns	Rf
1	02	0.15 0.06	03	0.26 0.15 0.06	02	0.15 0.06	02	0.15 0.06	04	0.41 0.26 0.15 0.06	04	0.41 0.26 0.15 0.06
2	02	0.17 0.00	03	0.36 0.17 0.00	02	0.17 0.00	02	0.17 0.00	04	0.45 0.36 0.17 0.00	04	0.45 0.36 0.17 0.00
3	02	0.18 0.01	03	0.48 0.18 0.01	02	0.18 0.01	02	0.18 0.01	04	0.63 0.48 0.18 0.01	04	0.63 0.48 0.18 0.01
4	02	0.07 0.00	03	0.16 0.07 0.00	02	0.07 0.00	02	0.07 0.00	04	0.34 0.25 0.07 0.00	04	0.34 0.25 0.07 0.00
5	02	0.26 0.12	03	0.37 0.26 0.12	02	0.26 0.12	02	0.26 0.12	04	0.45 0.37 0.26 0.12	04	0.45 0.37 0.26 0.12

S.S: Solvent system

NS: Number of spots.

Detection: modified Dragendorff's reagent

4: Gas Chromatography :

- The crude alkaloids are extracted and dissolved in chloroform, then injected in the gas Chromatography chamber under the following specifications:
- Column : length = 2 m, diameter 1/8"
- Stationary phase: 3% SE 30. Chromozore $\omega_1\omega$. ST 80%.
- Mobile phase: nitrogen at 60 ml/min.
- Column temperature: 260° C.
- Injection temperature: 270° C.
- Chart speed: 30 cm/h.
- Detector: flame ionization.
- Injected quantity: 2 µl.
- Technique: isotherm.

The result reveals the presence of four major alkaloids and one minor alkaloids. The R_F values are as follows:

1 → 0.375 min.

2 → 2.84 min

3 → 3.187 min.

4 → 4.325 min.

5 → 5.37 min.

Only four alkaloids appear in T.L.C. as shown in table 8.

5: Extraction and Isolation :

0.5 kg of powdered seeds are well dried and basified with 1.5 L of ammonia, so as to free the alkaloids from their salts. Then macerated with 5 L of ether during 12 hours. Ether solution is then drained with 9 L of hydrochloric water 2% into several fractions (during such operation, an emulsion is observed). The 2 phases are separated after 12 hours of decantation. The aqueous phase is again basified with ammonia and extracted with ether.

The ether solution (containing free alkaloids) is washed with water dried on Na_2SO_4 than evaporated at low pressure, providing 12.34 g of total alkaloids. After screening through T.L.C. a large non-alkaloidal spot is observed. A correction of alkaloids content is carried out by treating the total alkaloids with CH_2Cl_2 and hydrochloric water 2 %, again the aqueous solution is basified and extracted with ether an the total alkaloids are found to be 10.22 g (yield 2.04 %)

6: purification of alkaloids :

6-1: Purification by Column Chromatography :

The fractionating of alkaloids (crude) is conducted using column chromatography.

The crude alkaloids (500 mg) were dissolved into a minimum volume of $CHCl_3$ (3 ml) and set down over a column of silica prepared with $CHCl_3$. The elution is performed by means of chloroform and then a mixture of Chloroform and methanol with increasing polarity. The fractions are collected and analyzed through T.L.C. plates. The identical fractions are put together. The results give rise to four compounds (A, B, C, D)

The results are listed down in table 9 .

Table 9

Compound	Mass (mg)	%	U.V	Dragendorff	C.A.S	$FeCl_3/HClO_4$
A	627	10.16	Blue	Orange	Yellow	Pink/purple
B	3585	58.11	Blue	Orange	yellow-green	Purple/brown
C	1523	24.68	Blue	Orange	yellow-green	Purple/brown
D	434	7.03	Blue	Orange	yellow-green	Purple/brown

C.A.S = Ceric ammonium sulfate

	Color	R_f	R_t	Melting point
A	Brown reddish	0.63	2.840	234 - 238
B	White reddish	0.48	3.187	260 - 265
C	White reddish	0.18	4.325	230 - 235
D	White reddish	0.01	5.370	102 - 107

R_f: are retention factors using chloroform methanol (4:1)

R_t : are retention times using gas chromatography .

Melting points are not corrected.

Compound A: U.V. (λ_{max}^{meoh} nm): 223, 285, 293.

I.R. (cm^{-1}): 3060, 2980, 2920, 2845, 1695, 1450, 1370, 1220, 1150, 960, 810.

Compound B: U.V. (λ_{max}^{meoh} nm): 228, 284, 292.

I.R. (cm^{-1}): 3020, 2985, 2820, 1600, 1490, 1450, 1300, 1250, 1120, 960, 745.

Compound C: U.V. (λ_{max}^{meoh} nm): 225, 284, 291.

I.R. (cm^{-1}): 3060, 2980, 2830, 1610, 1490, 1405, 1320, 1240, 1150, 900, 740.

Compound D: U.V. (λ_{max}^{meoh} nm): 222, 281, 295.

I.R. (cm^{-1}): 3480 (large), 3260, 3060, 2840, 1700, 1480, 1360, 1360, 1210, 990, 810, 720.

6-2: Purification by planar chromatography [54] :

The purpose of this section has been to demonstrate that unmodified silica gel with buffered aqueous-organic eluents can be successfully applied to the separation of a great number of mixtures of organic bases, on both the analytical and preparative scales.

The alkaloids were eluted with methanol containing 10% 0.1 M hydrochloric acid. Mobile phase of constant ionic strength were prepared by mixing a constant amount of phosphate buffer of given pII, methanol and twice water.

Chromatography was performed on plates coated with 0.5 mm of silica gel. The adsorbent was suspended in phosphate buffer of appropriate pII. Saturation of chromatographic chambers was achieved by lining the tank walls with filter paper soaked in mobile phase.

The R_f values of alkaloids were determined as a function of volume fraction of methanol (ϕ)in the mobile phase, keeping the overall ionic strength and pH constant. The relationship obtained for pH 4, 5, 6 are presented in figures 1, 2, 3 at each pH value. R_m values :

$R_m = \text{Log}\,[(1 - R_f)/R_f]$ decrease with increasing methanol content, passing through a minimum when methanol content is 40 % and then increasing more or less sharply at high concentration of methanol. The exact position of the minimum $R_m = f(\phi$ methanol) relationship depends on the degree of polarity of alkaloids. The curves show that the methanol content is a very valuable parameter for adjusting the retention. It is also apparent from the influencing retention is to vary the pH.

In our experiment pH 6 was chosen as the best.

The chromatographic systems investigated can be used for the concentration of alkaloids from crude aqueous extracts. The methanol concentration or pH or a combination of these parameters can be used to adjust the order and degree of alkaloids. The proposed procedure has several advantages: Toxic chloroform is eliminated, and chromatograms can be used for micropreparative scale.

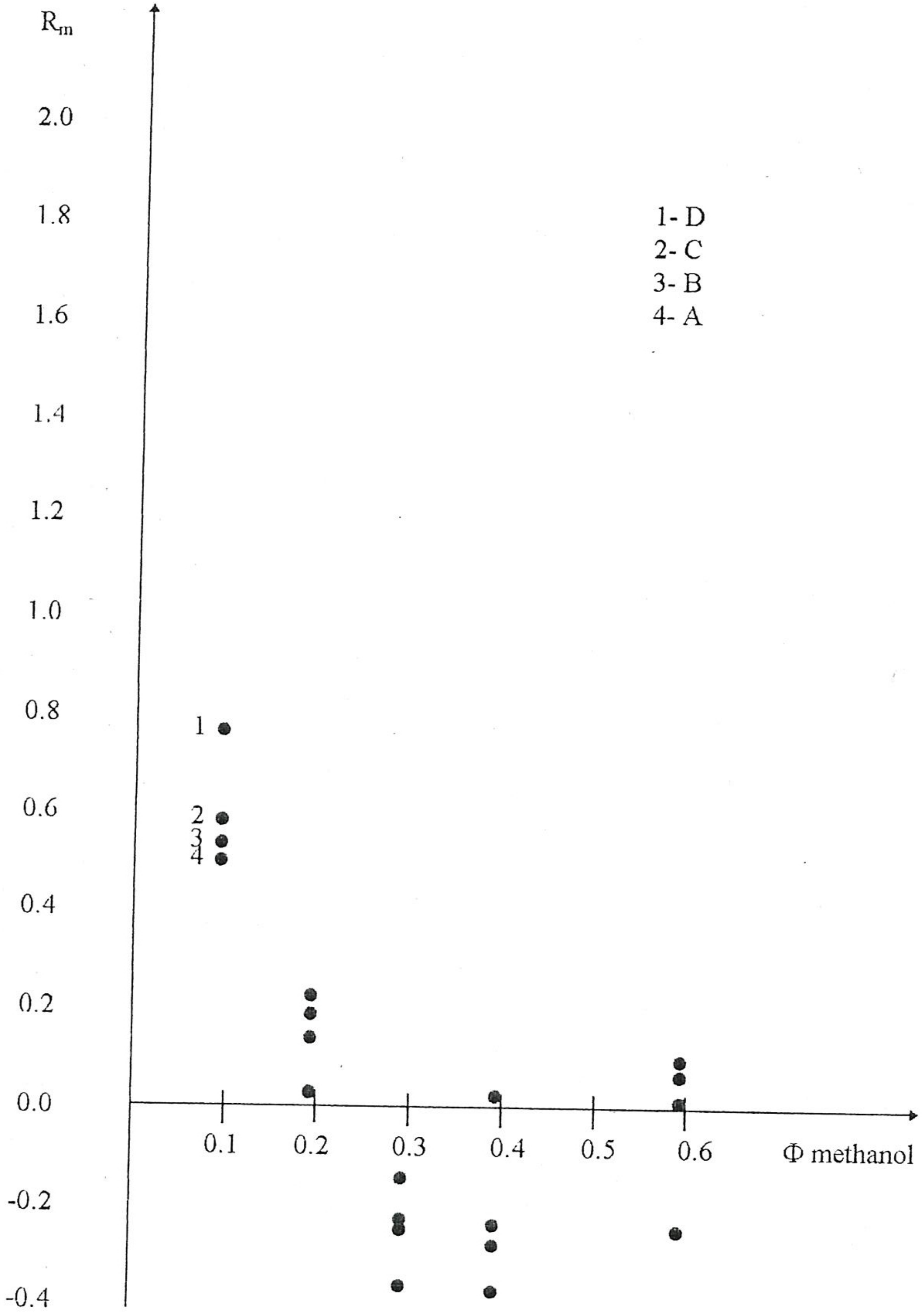


Fig :7 relationship between R_m values of alkaloids and the volume fraction of methanol in phosphate buffer (pH =4)

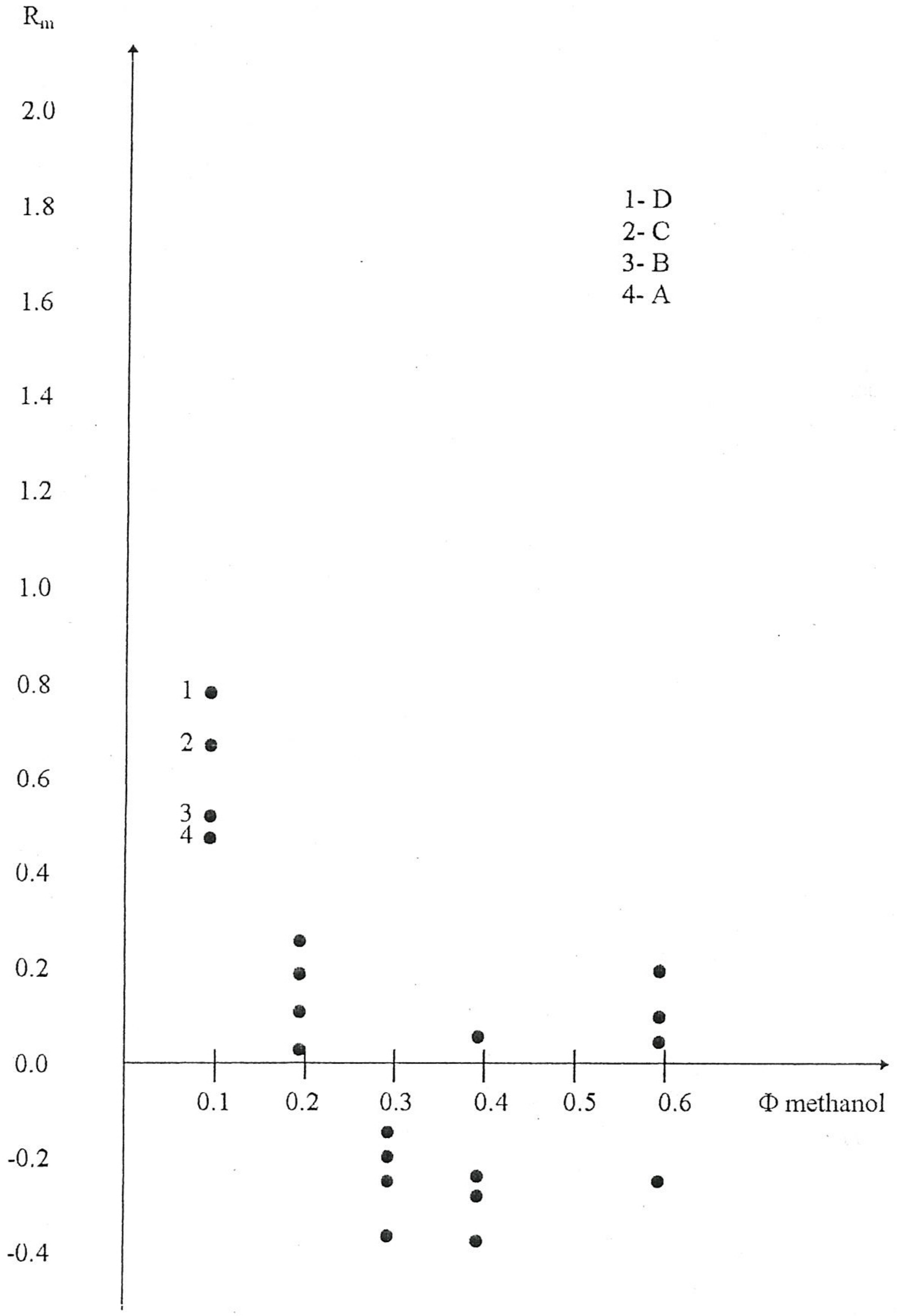


Fig. 8: relationship between R_m values of alkaloids and the volume fraction of methanol in phosphate buffer (pH =5)

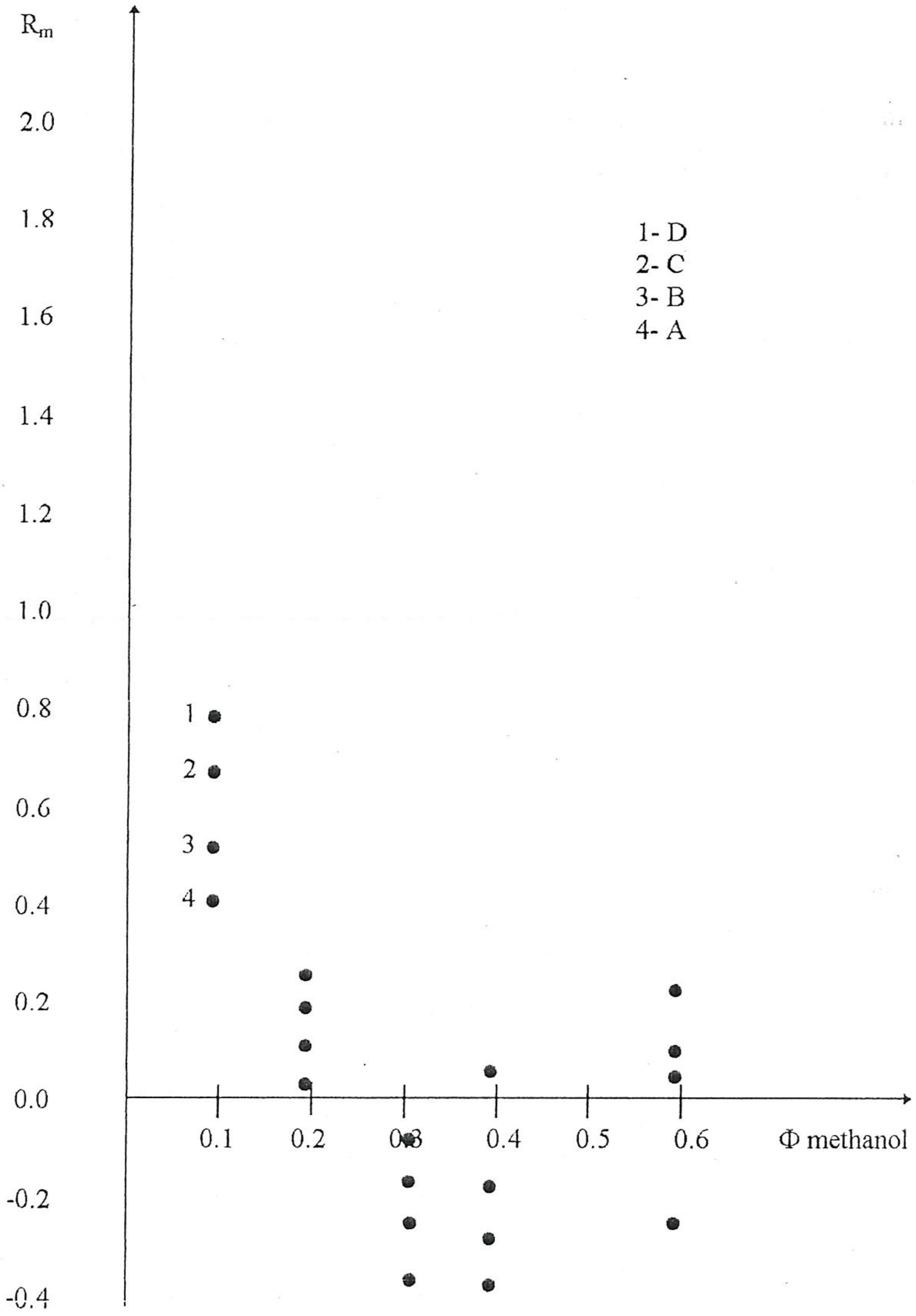


Fig.9: relationship between R_m values of alkaloids and the volume fraction of methanol in phosphate buffer (pH =6)

7: Discussion and Conclusion:

The objective to be achieved through this study was to determine the alkaloidal composition of "Peganun Harmala" both qualitatively and quantitatively.

This plant - famous for its curative properties- has not been studied so far, in spite of its wide use in traditional medicine. Moreover the project was intended to provide a quite useful and basic tool for any further investigation regarding the effect of Harmala alkaloids in pharmacology and biochemistry.

The plant parts are collected throughout the year and tested for the presence of some familiar natural compounds. The results revealed the presence of oils, Saponins, glycosides, alkaloids, tannins and ketoses. Since we are primarily interested in alkaloids, we have opted to compute the amount present in different parts, through successive extractions, using four different solvent systems. The best results are shown when using ether after moistening the powdered plant material with ammonia and the maximum content was found in the seeds (4.21 %).

Once the crude alkaloids are in hand, the next step is to determine the best solvent system to be used for an appropriate separation. For this sake, 20 solvent systems have been tried in thin layer chromatography and the results for the best five ones are listed down in table (8) page (50). The mixture of chloroform and methanol was chosen for the subsequent purification owing to its consistent R_F values. Almost all the solvent systems in T.L.C. give rise to four spots and hence four compounds.

Gas liquid chromatography confirmed the presence of five compounds among which one could not be identified in T.L.C. because of its minor amount. The other four peaks come one after the other according to their molecular weights, polarity and retention times found in the literature [55, 56], we immediately assigned the first peak as Harmane (50) (MW = 182), the second peak as Harmine (51) (MW = 212), the third peak as

Harmaline (54) (MW = 214), and the fourth peak was assigned to be Harmalol (55) (MW = 200).

Harmane: melting point: (234 - 238°C) → literature [57, 60] (235 - 238°C)

Harmine: melting point: (260 - 265°C) → literature [57, 60] (263 °C)

Harmaline: melting point: (230 - 235°C) → literature [57, 60] (232 - 234°C)

Harmalol: melting point: (102 - 105°C) → literature [57, 60] (100 - 105°C)

The infrared and ultraviolet spectra are almost the same as those found in the literature [58, 60, 61].

This work has shown:

a) The presence of alkaloids in Algerian Harmala.

b) Alkaloids are present mainly in the seeds and in quantities no less than those found in the same plant in other regions of the world. This analysis gave (4.21%) of alkaloid content in the seeds.

In Germany	→	(3.8 - 5.8 %)[33].
In Russia	→	(3.01%)[37].
In Pakistan	→	(4.5%)[54].

The Harmala alkaloids have a lot of pharmaceutical effects: they can be dissolved in water and drunk to relieve intestinal pain and kill parasites. They can as well be used in form of compress around the aching part of the body. In the vapor form they are very active as insecticides [38, 40, 41, 42].

Our intention in the future is to try to work with other people in the field of pharmacology to confirm those curative properties.

We intend as well to synthesize these products whose healing effects were proven.

We feel deeply indebted as the N.M.R. work was not achieved including ^{15}N n.m.r. where the method is available [59] and this because we had no way of doing this work as we could not get access to this technique in Algeria and have not benefited from any short training in any chemistry laboratory abroad. This remains one of our major concerns and will hopefully be done as soon as the occasion is available.

Appendix

Alkaloid detecting reagents

Precipitation

Mayer's Reagent

Mercuric chloride	1.36 g
Potassium iodide	5.00 g
Water to	100 ml.

Wagner's Reagent

Iodine	1.30 g
Potassium iodide	2.00 g

Water to 100 ml.

Dragendorff's Reagent (Kraut Modification)

Bismuth nitrate	8.00 g
Nitric acid	20.0 ml
Potassium iodide	27.2 g
Water to	100 ml.

Chromatography

Antimony (III) Chloride

25 g of antimony (III) chloride in 75 g of chloroform. Heated for 10 min at 100° C after spraying.

Ceric Ammonium Sulphate

1 g of cerium is dissolved in 99 g of 85% phosphoric acid by heating the mixture on a hot plate for 5 - 10 min., the spray reagent can be used as such or after dilution with an equal volume of water, in which case, however more than one spraying may be necessary.

Ceric sulphate - Sulphuric Acid (Sonnensch Reagent)

0.1 g of ceric sulphate suspended in 4 ml of water; add 10 ml of trichloroacetic acid; heat; add concentrated sulphuric until turbidity disappears.

Iron III chloride, perchloric acid

1 ml of 0.5 m iron (III) chloride solution is mixed with 50 ml of 35% perchloric and the chromatogram is observed immediately after spraying after heats with hot air and after heating at 110° for 30 min.

Iodoplatinate Reagent

3 ml of 10% hexachloroplatinic (IV) acid solution in 97 ml of water mixed with 100 ml of 6% potassium iodide solution in water, the reagent should be freshly prepared.

Dragendorff's reagent (Bregoff - Delwiche)

(I) 8 g of bismuth subnitrate is dissolved in 20 ml of 25% nitric acid.

(II) 20 g of potassium iodide is dissolved in a mixture of 1 ml of 25% hydrochloric acid and 5 ml of water. I and II are mixed and water is added until an organ-red colour is obtained (the volume is about 95 ml). The solution is filtered and diluted to 100 ml with water. The spray reagent is prepared by subsequently mixing 20 ml of water, 5 ml of 6 M hydrochloric acid, 2 ml of stock solution and 6 ml of 6 M sodium hydroxide solution. If bismuth hydroxide is not completely dissolved by shaking, 6 M hydrochloric acid is added until a clear solution is obtained. The stock solution is storable for longer periods; the spray reagent is stable for 10 days if stored cool.

Dragendorff's reagent (Munier)

(I) 1.7 g of bismuth subnitrate is dissolved in 100 ml of 20% tartaric acid solution.

(II) 16 g of potassium iodide are dissolved in 40 ml of water. I and II are mixed. The spray reagent is prepared by mixing 50 ml of the stock solution, 100 g of tartaric acid and 500 ml of water.

Dragendorff's reagent (Munier and Macheboeuf)

Preparation:

(I) 2.5 g of bismuth subnitrate are dissolved in a mixture of 20 ml of water and 5 ml acetic acid.

(II) 4 g of potassium iodide are dissolved in 10 ml of water.

I and II and are mixed. This stock solution can be stored for at least 6 months. The spray reagent is prepared by mixing 5 ml of the stock solution with 10 ml of acetic acid and dilution to 100 ml with water.

Dragendorff's reagent (Robles)

5 g of potassium iodide are dissolved in a mixture of 12.5 ml of water and 3 ml of 4 M hydrochloric acid. 1 g of bismuth nitrate is dissolved in this solution by boiling. After cooling, 12.5 ml of water are added. To this solution 5 g of potassium iodide and 5 g of citric acid are added. The solution is further diluted with 75 ml of 0.1 M hydrochloric acid.

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ملخص

يعتبر نبات الحرمل من أهم الأعشاب الطبية المنتشرة بكثرة في الشرق الجزائري وهو معروف بخصائصه العلاجية وقدرته على تسكين الآلام المترتبة عن مرض الروماتيزم و القضاء على الطفيليات التي تتخذ من الأمعاء مقرا لها. لكن بالرغم من الأهمية التي يكتسيها هذا النبات، لم تتم دراسة المواد الفعالة التي يحتويها ولو بشكل تقريبي الشيء الذي حفزنا على المضي في هذا المشروع من أجل تحديد تكوين هذا النبات فيما يتعلق بالقلويدات كما و كيفا في مختلف أجزائه وعلى مدار السنة ومقارنة هذه النتائج مع النتائج المتحصل عليها في مختلف أنحاء العالم. و نهدف زيادة على ما سبق ذكره إلى إيجاد طرق ووسائل بسيطة لإستخلاص و عزل القلويدات و توفير معطيات هامة لأي دراسة نباتية أخرى في مجال البحث على المواد الفعالة.

وترتكز هذه الدراسة على ثلاثة محاور .

المحور الأول : ويحتوي على دراسة مستفيضة للقلويدات سواء تعلق الأمر بالتسمية أو بالتصنيف أو طرق الإستخلاص أو المنافع الطبية .

المحور الثاني : ويحتوي على الوصف الفيزيولوجي للحرمل مع تصنيفه في مملكة النبات.

المحور الثالث : ويمثل الجزء التجريبي ويحتوي على مختلف إختبارات وجود المواد الفعالة النباتية كالزيوت الطيارة، الغليكوزيدات، الصابونينات، الطانينات، ... الخ.

كما يشمل أيضا مختلف طرق الأستخلاص وأنظمة المذيبات التي أعطت نتائج معتبرة وأخيرا التعريف بالقلويدات المستخلصة .

لقد بينت الدراسة وجود أربعة قلويدات بكميات متفاوتة في الحرمل ، هذه القلويدات التي بالمقارنة مع ماهو متوفر لدينا من مراجع نسبت إلى:

الحرمن ، الحرمين ، الحرملين و الحرملول وهي موجودة في معظم أجزاء النبات خلال المراحل المختلفة من نموه .كما بينت الدراسة أن أعلى نسبة للقلويدات موجودة في البذور وأن الطريقة المثلى لأستخلاصها هي بإستعمال الاثير . وفيما يتعلق بأنظمة المذيبات فقد تبين أن خليط من الكلوروفورم ، و الميثانول أعطى نتائج جيدة وقد أعتمد عليه أثناء عملية العزل في العمود الكروماطوغرافي .كما أعتمدت طريقة أخرى لفصل القلويدات ترتكز أساسا على خليط من الميثانول ومحلول حمضي بأستعمال كروماطوغرافيا الطبقة الرقيقة وذلك بتثبيت pH المحاليل المستعملة ولوحظت أحسن نتيجة عند pH = 6 .

RESUME

Le Peganun Harmala est une plante médecinale très répandue dans l'Est de l'Algérie . Elle est connue par ses effets thérapeutiques et son efficacité pour soulager les douleurs rhumatismales et attaquer les parasites intestinals. Malgré l'importance qu'engendre cette plante, son étude n'a pas été réalisée en Algérie jusqu'à nos jours. Ce projet , donc , a pour objectif de déterminer la composition de la plante en terme de principes actifs (alcaloïdes) aussi bien qualitativement que quantitativement et la comparer avec les résultats obtenus dans d'autres régions du monde .

Notre but , aussi, a été de développer des méthodes simples pour les procédés d'extraction et d'isolution des alcaloïdes et de fournir des données de base nécessaires pour d'éventuelles recherches dans le domaine des substances naturelles .
Notre étude est composée de trois parties:

La première partie *se rapporte à une étude générale des alcaloïdes y compris: La nomenclature, la classification, les méthodes de séparation et la pharmacologie.*

La deuxième partie *comprend une description physiologique et taxinomique de la plante ainsi que son systématique dans le règne végétale.*

La troisième partie *(expérimentale) est un exercice de chimie des substances naturelles et comprend essentiellement les tests de présence de quelques composés naturels à savoir : Les huiles volatiles, les glycosides, les saponins, les cardenolides, les tannins,...etc, ainsi que les procédés d'extraction et d'isolation en utilisant des différents modes de solvant pour une meilleure séparation et enfin l'identification des alcaloïdes obtenus.*

En se basant sur les données physiques disponibles dans la littérature, l'étude a révélé la présence de quatre alcaloïdes en quantité plus au moins importante dans les différents organes analysés à savoir: Harmane , Harmine , Harmaline et Harmalol. Le taux maximum des alcaloïdes à été trouvé dans les grains en utilisant l'éther dans la phase d'extraction .

Parmi les divers solvants utilisés, le mélange chloroform-methanol a donné de bons résultats et il est utilisé donc pour la purification sur colonne . Une méthode de purification à l'échelle semi-préparative a été développée en utilisant comme éluant le méthanol dans une solution aqueuse d'acide à force ionique constante (pH). Les meilleurs résultats sont observés à pH = 6 .