Summary

In this work two Iraqi plants (*Datura Stramonium* and *atropa belladonna)* has been studied. Extraction and isolation of atropine from these plants were investigated. The extraction was done using methanol as a solvent using soxlet apparatus. Atropine was identified by melting point which was found to be (113-116 ^{o} C) in *Atropa belladonna* and (114-118^{o}C) in *Datura stramonium* which was in good agreement to the standard value (114-116C^{0}). F.T.IR, UV-Vis, HPLC and TLC achieve the presence of atropine.

HPLC analysis shows New Flavonoid compounds in the two plants in small concentrations. Rutin and Qurecetin were separated in *Datura stramonium* while Catechin was found in *Atropa belladonna.* TLC also confirmed the presence of these compounds by comparison the R_f values of each extracted compounds with the standard R_f values. Biological activity was determined using disc diffusion method with two strain of pathogenic microorganisms viz., *Staph.aureus* and *E.coli.* These results obtained using different water extract concentrations (5µg/ml, 10µg/ml, 15µg/ml, and 20µg/ml) showed measurable activity against the two types of bacteria.

Acknowledgment

Praise to ALLAH the first cause of all causes, the glorious creator of the universe and blessing up on Mohammad Profit of God and up on his family.

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Finally to all my friends I present my thanks.

Bassam

2005

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

وَيَسْـَٔلُونَكَ عَنِ ٱلرُّوحِ ۖ قُلِ ٱلرُّوحُ مِنْ أَمْرِ رَبِّى

وَمَآ أُوتِيتُم مِّنَ ٱلْعِلْمِ إِلَّا قَلِيلًا ﴿٨٥﴾

صدق الله العلي العظيم
الإسراء-٨٥

Aims of this work

1. The aim of this work was to extract and isolate atropine compound from two Iraqi plants (*Datura stramonium, Atropa Belladonna)* and determining the weight of atropine in each of those plants.

2. The second aim of this work was to detect presence of other biologically active compounds.

3. The third aim was to investigate the biological activity of the aqeous extract of the two plants in different concentration against some kind of bacteria.

A. *Atropa Belladonna*:

3. A.1.Basic constituents:

Chemical tests that applied on the Herb are shown in Table (1). The first test was the determination of the pH which was found equal to 5.31. This value indicated the low acidity of the water extract of herb which may be due to the alkaloid content of the extract. Saponin test gave positive result which indicated by the appearance of foam. The presence of tannins indicated by appearance of gelatinous precipitate, this precipitate considered as a positive result. Resin also tested and gave positive result by appearance of turbid solution. Three tests were done for alkaloids which is (Dragendrof, Mayer and Wagner) in which all of these testes gave positive results by appearance of orange, brown and yellow precipitate respectively. Positive result was also found for Flavonoid test by appearance of yellow precipitate.

Table (1) Tests applied on *atropa Belladonna*

Test	Result
Determination of Acidity(pH)	**5.31**
Saponins test	**Positive**
Tannins test	**Positive**
Resins test	**Positive**
Alkaloids test	
Dradgndroff	**Positive**
Wagner	**Positive**
Mayer	**Positive**
Flavonoids	**positive**

3. A.2.Atomic absorption measurement:

The concentrations of mineral elements found in the leaves of *atropa belladonna* are shown in Table (2).The result of those measurement differ from that published by Szoke [78]. Those differences may due to many reasons such as environmental differences due to nature and composition in different location. The very low level of heavy metals (Cu, Mn and Zn) and the highly level of nutrient elements (Ca, K, Fe and Mg) make it useful for treatment in popular medicine.

Table (2) Atomic absorption measurements of solution of *Atropa belladonna*

Element	Concentration in ppm
Cd	**0.00**
Cu	**0.7**
Mn	**1.2**
Zn	**1.0**
Fe	**50.1**
Mg	**50.3**
Ca	**470.0**
K	**550.0**

3. A.3.Atropine extraction

Crude alkaloids were obtained under procedure shown in (2.8). Atropine obtained from crude alkaloids. Three experiments were made using 8 gm from the plant, the net result was found to be 0.0293 g, with percentage equal to 0.366%. This value differ from that found by Swain [14] which was equal to 0.45%.

Table (3) The amount of crude atropine that obtained

No. of exp.	Weight of Herb	Weight of atropine	Average weight	%
1	8 g	0.033 g	0.0293 g	0.366%
2	8 g	0.0296 g		
3	8 g	0.026 g		

3. A.4.Identification of atropine:

3. A.4.1.Melting point:

Melting point was measured and found to be 113-116^{o}C, this value identical with the published value (114-116^{o} C) [68]

3. A.4.2. UV-Vis measurements:

Ultra-violet spectrum was measured in ethanol as a solvent. Figure (2) shows the maximum absorption for atropine which is equal to (283.1nm) indicates a small difference with that of standard solution peak shown in figure (1) [73] that equal to (285nm).

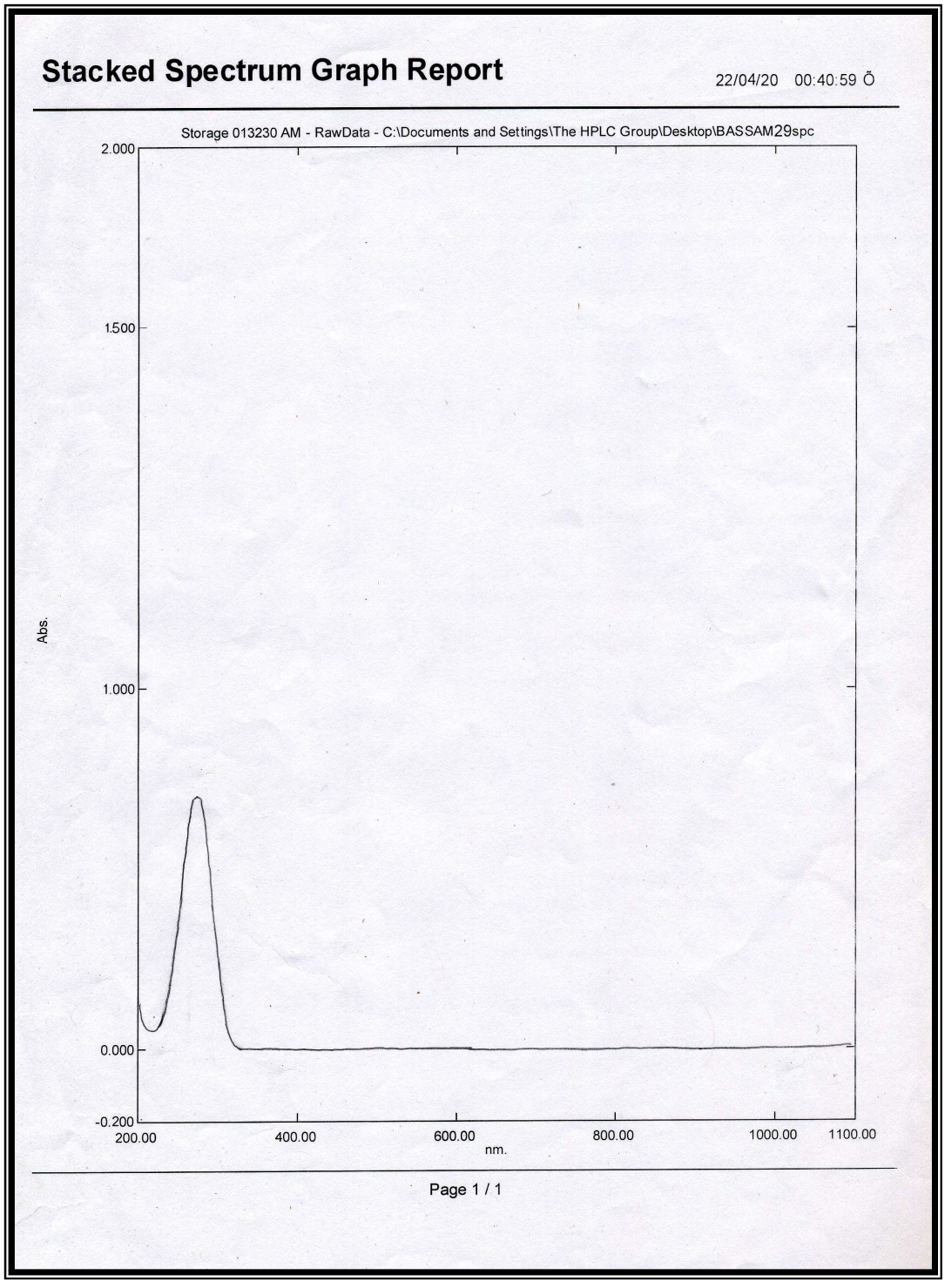


Figure (1). UV spectra for standard atropine solution

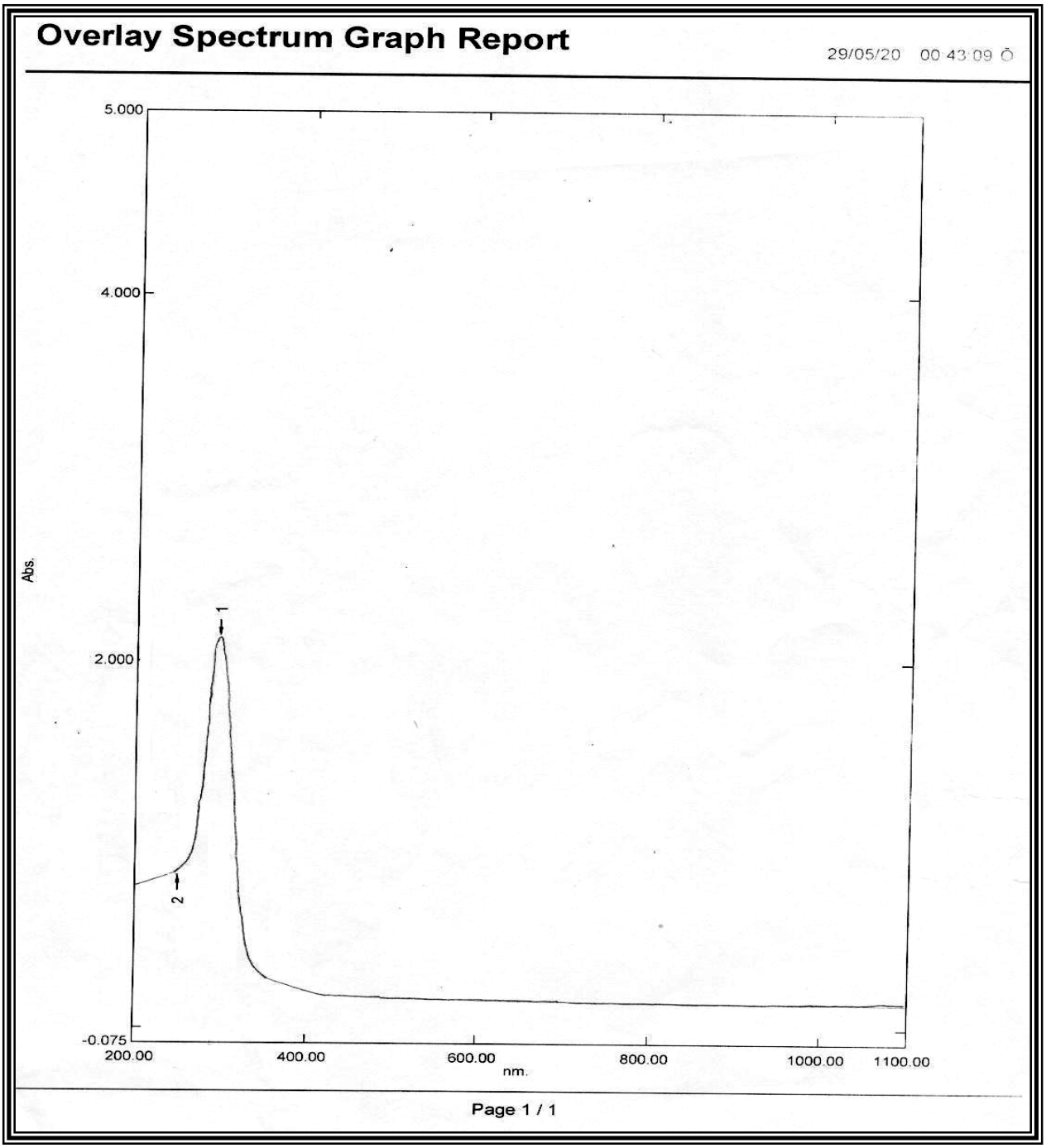


Figure (2) UV spectra for atropine that extracted from *Atropa belladonna*

3. A.4.2.HPLC:

Chromatographic separation of the studied samples with HPLC were measured using Tetrahydrofuran: deionizedwater: acetic acid in (35:65:1) v/v as a mobile phase. Atropine standard was injected in HPLC in 5µg/ml as a concentration. The retention time for atropine was found to be 11.18 min . A 1.0 gm from crude aqueous extract were dissolved in 10 ml of methanol and injected in HPLC. The retention time of atropine in extract have retention time equal to 11.15 min with peak area 114084. Figure (3) shows HPLC chromatogram for the extracted atropine.

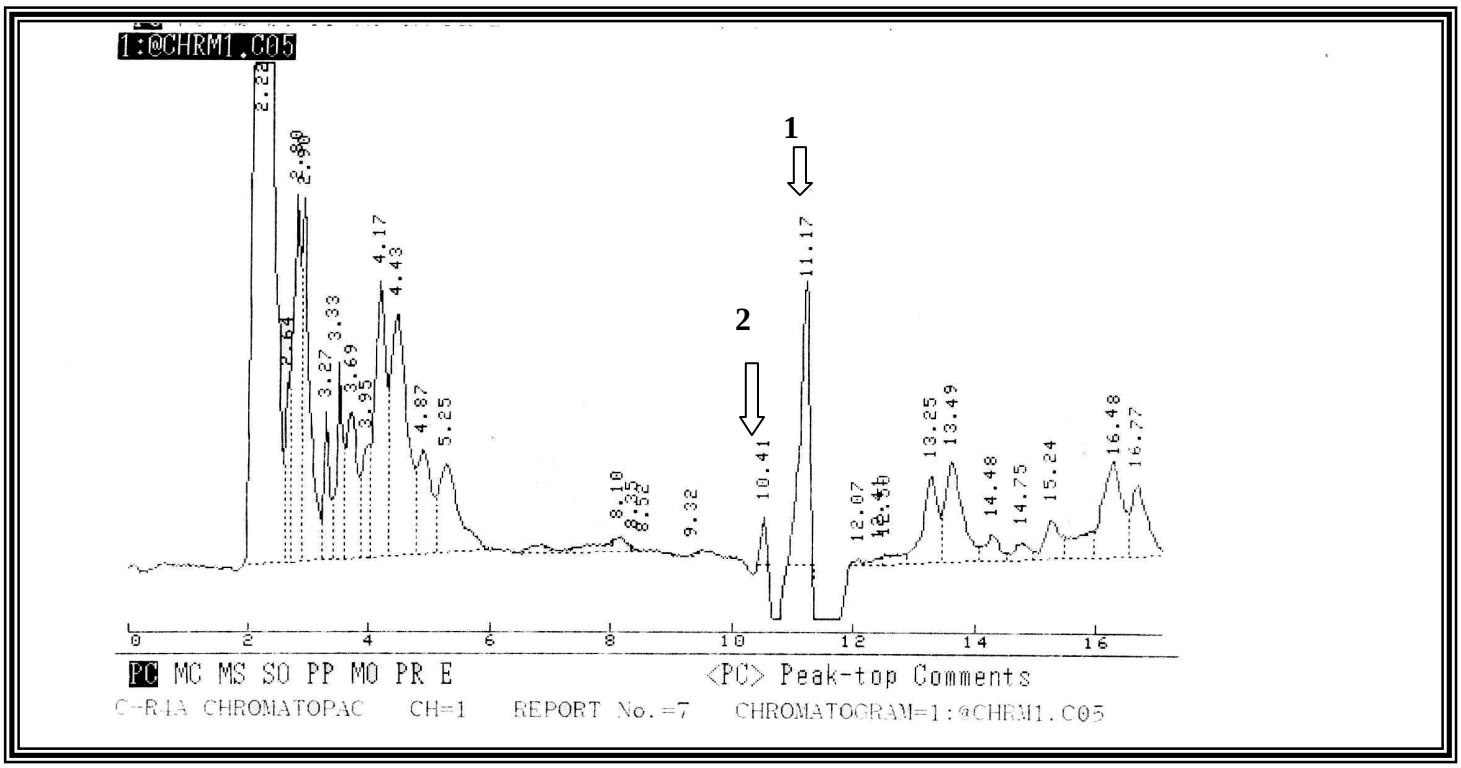


Figure (3) HPLC measurement for total extract of *atropa belladonna*
1 (atropine)
2 (Catechin)

3. A.4.4. TLC:

Atropine was identified by TLC as described in (2.2.6.).Two different solvent systems were gave the best results:

a. n-Butanol/acetic acid /distilled water (4:1:5)[46]
b. water/ acetic acid (8:2)[46]

The R_f values for standard atropine in solution a and b were found to be (0.74 and 0.82), respectively while the R_f value for atropine that extracted with both solution a and b were found to be (0.72, 0.81), respectively. Detection was done using UV radiation which gave bright blue coloration.

TLC analysis shows that the compound that isolated was atropine by comparison with the value of R_f of the standard solution.

Table (4) Identification of atropine using TLC with two solvent systems.

Atropine (standard)	**R_f**	**Colour in UV radiation**
Solvent system(a)]	**0.74**	**bright blue**
Solvent system (b)	**0.82**	**bright blue**
Atropine (extracted)		
Solvent system (a)	**0.72**	**Bright blue**
Solvent system (b)	**0.81**	**Bright blue**

3. A.4.5. F.T.IR

The values of standard peaks taken from standard spectrum is shown in figure (4) as published by (SCHEINMAMN)[1970] Figure (5) shows the F.T.IR spectrum for the extracted atropine

Table (5) F.T.IR measurement for major bands absorption for standard and extracted atropine

Functional group	**Peak value for standard atropine 1\cm**	**Peak value for extracted atropine 1\cm**
C-H aromatic	**3095**	**3020**
C-H aliphatic	**2930-2800**	**2866**
O-H	**3500-2700**	**3388.7**
C=O	**1720**	**1712.7**
C=C	**1600**	**1530**
C—O—C	**1245-1110**	**1245.9-1110.9**

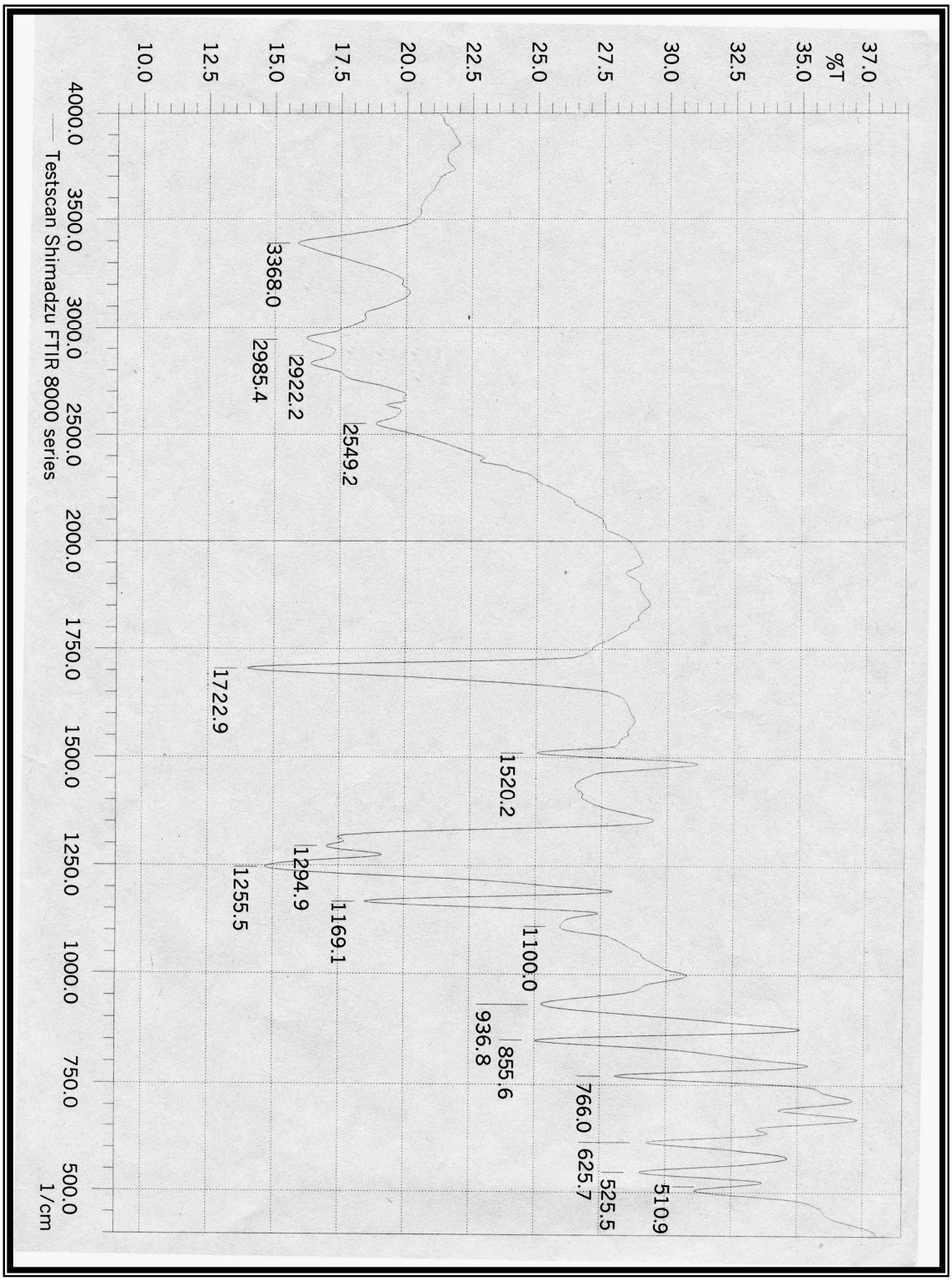


Figure (4) F.T.IR measurement for standard atropine

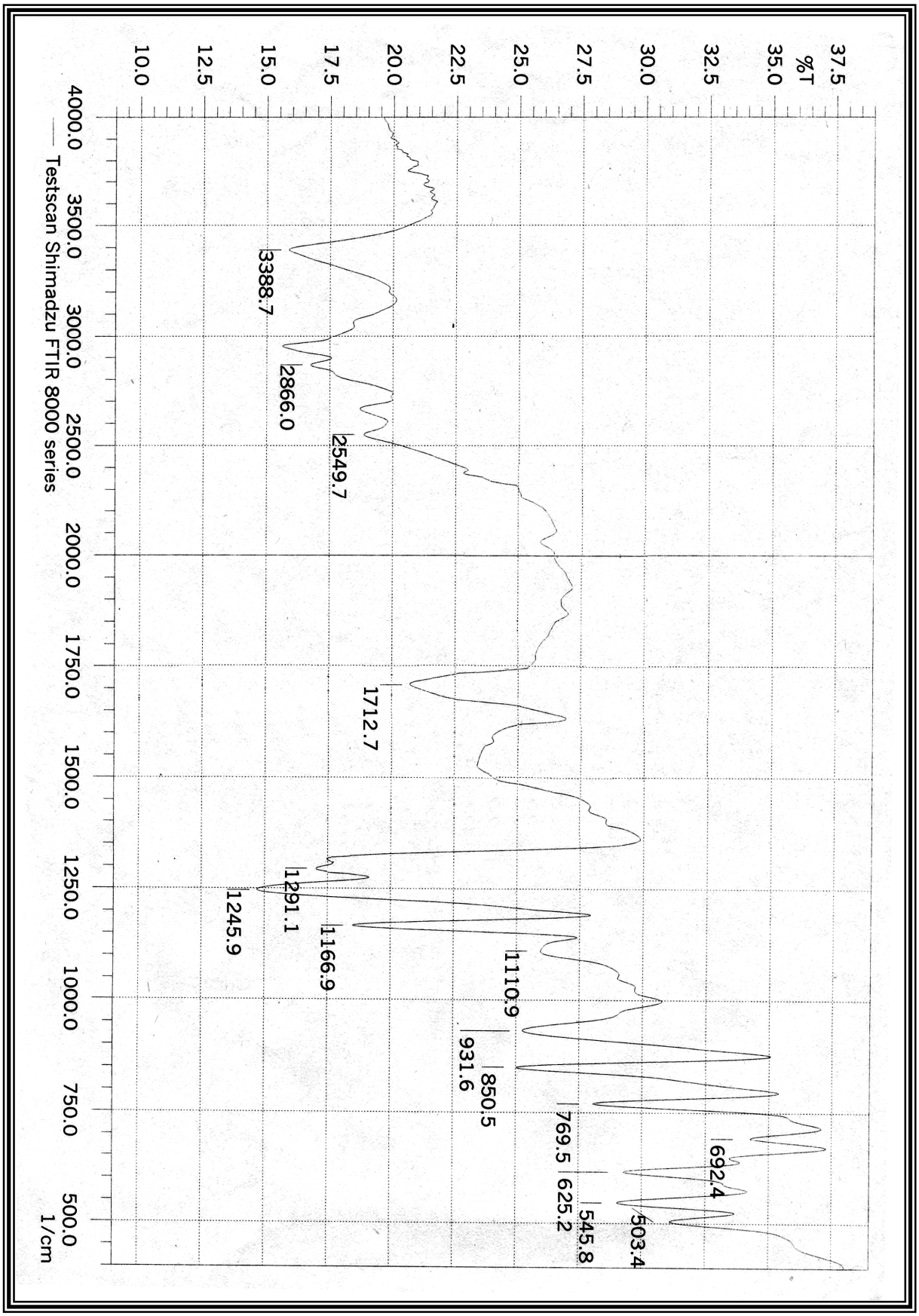


Figure (5) F.T.IR spectra for atropine that extracted from *atropa belladonna*

3. A.5.Flavonoids

3. A.5.1.H.P.L.C

The HPLC analysis was done using tetrahydrofuran: deionized water: acetic acid (35:65:1)[77] v/v as a mobile phase. A 5µg/ml concentration of standard solution of different Flavonoids was injected in HPLC. The retention time of standard catechin was found to be (10.46 min) and that in total extract was found to be (10.47 min) with peak area (4216). Figure (3) shows the HPLC chromatogram for the total extract of *atropa belladonna*

3. A.5.2.Flavonoids extraction:

Two experiments were made by taken 10 g from the herb. The net weight of total Flavonoids was found to be 3.5 g with percentage of 0.035%.

3. A.5.3.T.L.C

T.L.C analysis was done using two solvent systems;

a-ethylacetate/formic acid/methylethylketon/water (5:1:3.5:0.5)[63]

b-toluene/chloroform/acetone (40:25:35) [63]

The R_F value for standard catechin using solutions (a) and (b) were found to be (0.35 and 0.75) respectively and for that found in crude flavonoids were R_f (0.32 and 0.78), respectively as shown in table (6)

Table (6) the R_f values for atropine

	R_f	**Detection in UV**
catechin(standard)		
Solvent system(a)]	**(0.35)**	**bright yellow**
Solvent system (b)	**(0.75)**	**bright yellow**
catechin(in crude flavonoid)		
Solvent system (a)	**(0.32)**	**bright yellow**
Solvent system (b)	**(0.78)**	**bright yellow**

B. *Datura stramonium*

3. B.1 Basic constituents:

Chemical tests that applied on the herb are shown in Table (7) below. The first test for determination of the pH which was found to 6.46. This value indicated a low acidity of the water extract of herb which may be due to the alkaloid content of the extract. This value is larger than the pH in *Atropa belladonna.* This result is in agreement with that of the *Datura stramonium* which contained a larger amount of atropine than in *Atropa belladonna*. Saponin test gave positive result by the appearance of foam as described in

(2.7.4). Tannins test gave negative result which is in agreement with the published result by Maldonado [46]. Resin also tested and gave positive result by appearance of turbid solution. Three tests were done for alkaloids which is (Dragendrof, Mayer and Wagner). All of these tests gave positive results by appearance of orange, brown, and yellow precipitate, respectively. Positive result found for Flavonoid test by appearance of yellow precipitate.

Table (7) Tests applied on *Datura stramonium*

Test	**result**
Determination of Acidity (pH)	**6.45**
Saponins	**Positive**
Tannins test	**Nagitive**
Alkaloids test	**Positive**
Dradgndroff	**Positive**
Mayer	**Positive**
Wagner	
Flavonoids	**positive**

3.B.2 Atomic absorption measurement:

Table (8) show the concentrations of mineral element in the seed of *Datura stramonium.* The results of those measurements comparable to that published by Ballica.R [79]. The very low level of heavy metals (Cd, Mn, Zn) and highly level of nutrient elements (Ca, K, Fe and Mg) make it useful for treatment in popular medicine. In this side the herb need further chemical and biological studies.

Table (8) atomic absorption measurements of extract

Element	Concentration in (ppm)
Cd	**0.00**
Cu	**0.8**
Mn	**1.5**
Zn	**1.5**
Fe	**40.1**
Mg	**46.3**
Ca	**480.0**
K	**450.0**

3. B.3. Atropine extraction

Two experiments were made using 7g for each. The average result was found equal two 0.0327g with 0.46% of the weight of the seed.

3. B.4. Identification of extracted atropine that extracted:

3. B.4.1.Melting point:

Melting point was measured and found to be 113-118 C^0, this value identical with the published value (114-116 C^0) [68].

3. B.4.2.UV-Vis measurements:

Ultra-violet spectrum were measured according to (2.2.) in ethanol as a solvent. Figure (6) shows the peak for atropine that equal to 282.3 nm this value comparable to the standard peak that equal to 285 nm [73].

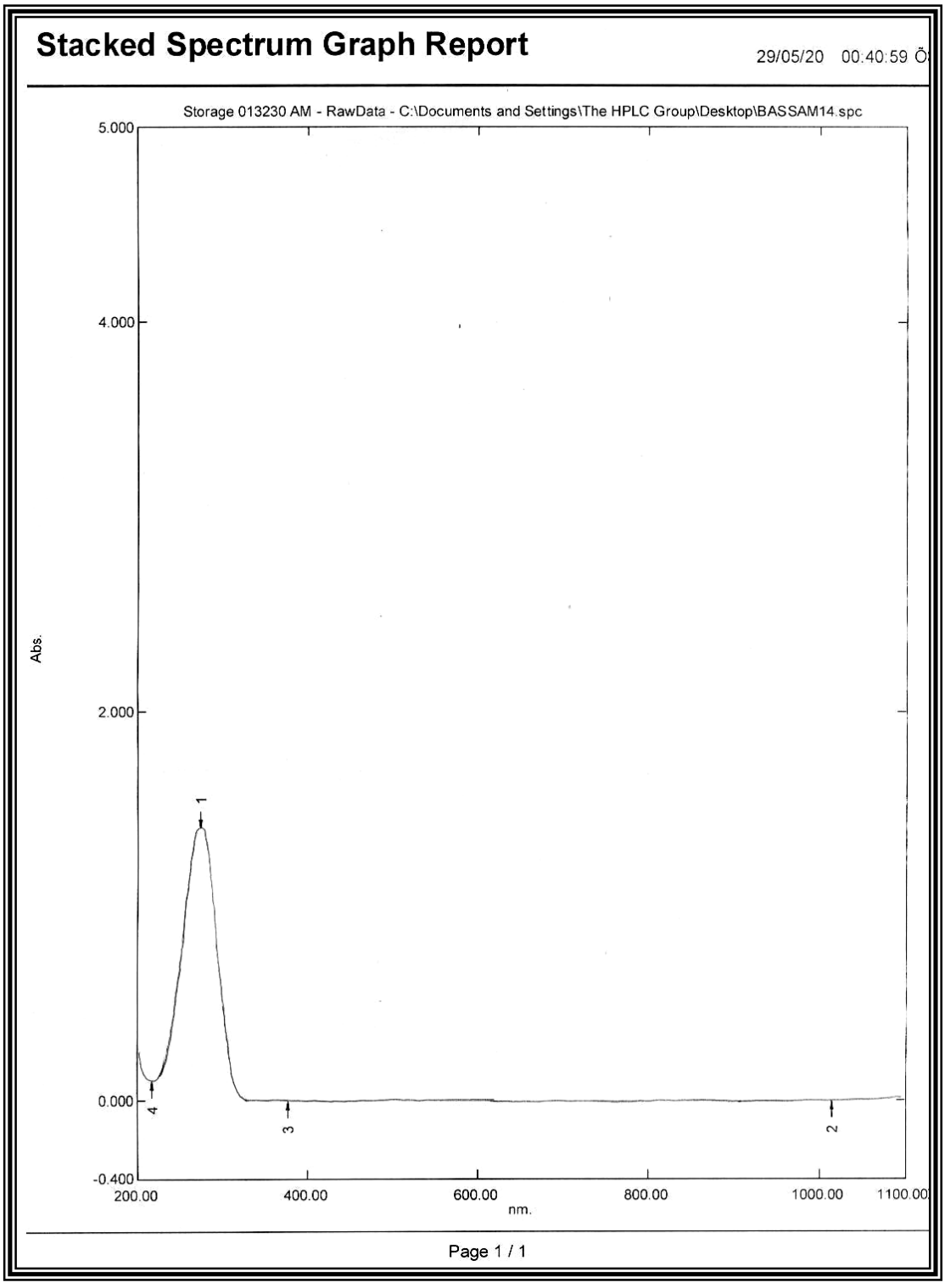


Figure (6) UV spectra for atropine that extracted from *datura stramonium*

. 3.B.4.3. HPLC analysis:

HPLC separation was used with Tetrahydrofuran: deionized water: acetic acid in (35:65:1)[77] v/v percent as a mobile phase. Atropine standard 5µg/ml was injected. A 1.0 gm from crude aqueous extract were dissolved in 10 ml of methanol and injected in HPLC.The standard atropine shows retention time at 11.15 min. while for the total extract shows retention time equal to the retention time of standard atropine this value equal to 11.16 min as shown figure (7).

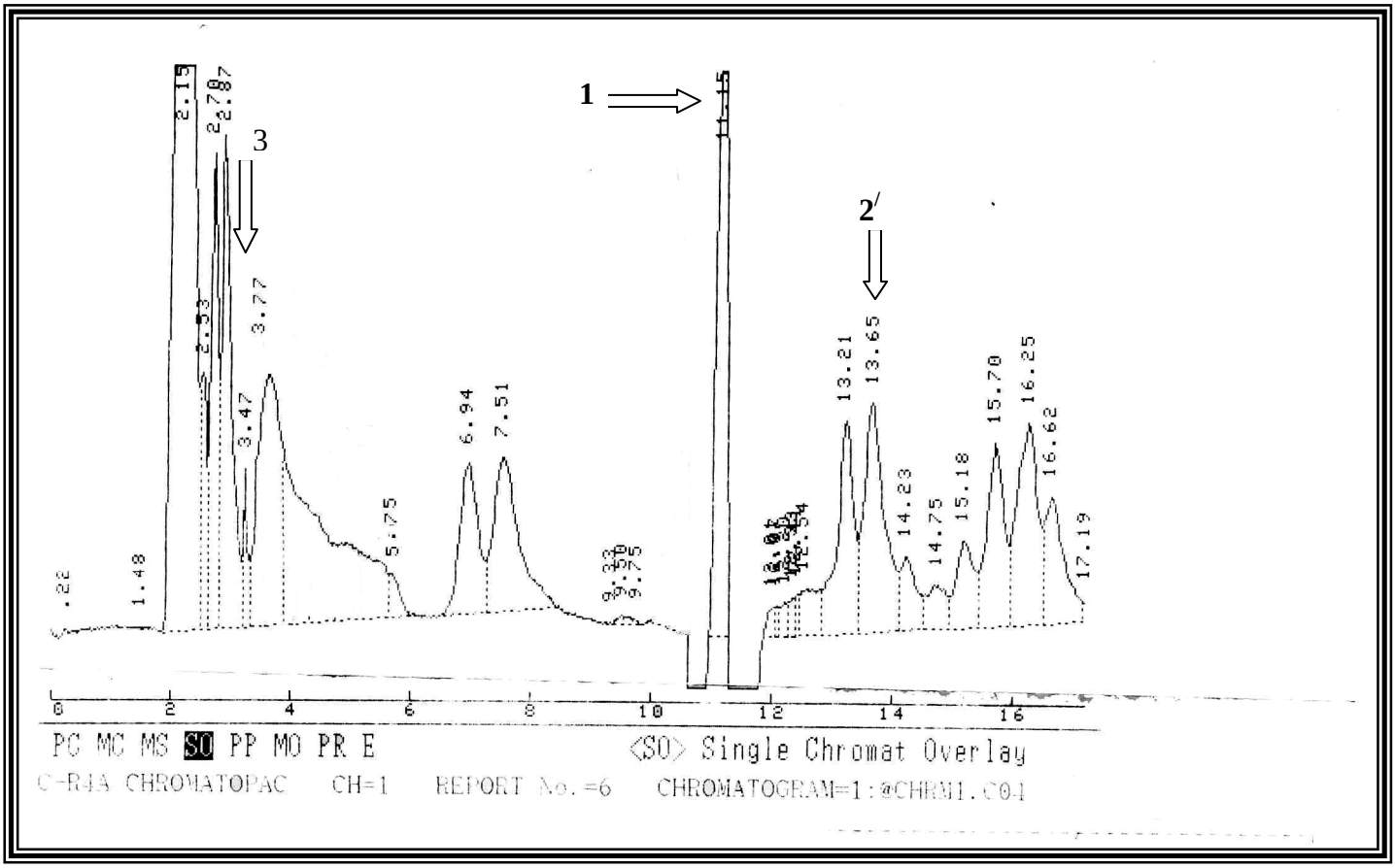


Figure (7) HPLC measurement for total extract of *Datura stramonium*

1 (Atropine), 2/ (Querecitine) , 3 (Rutin)

. **3. B.4.4.TLC**:

Atropine was identified by TLC as described in (2.6.), different solvent systems were used in this study. Two different solvent systems were gave best results:

a. n-Butanol/acetic acid/ distilled water (4:1:5)[46]

b. water/ acetic acid (8:2) [46]

TLC shows that the compound that isolated is atropine by comparison the of R_f values of standard which were equal to 0.74 and 0.82 in solution a and b respectively while the R_f values for atropine that extracted from the Herb were equal to 0.75 and 0.80 for solution a and b respectively. As shown in table (9).

Table (9) identification of atropine using TLC with two solvent systems

	R_f	**Color in UV radiation**
Atropine (standard)		
Solvent system(a)	**(0.74)**	**bright blue**
Solvent system(b)	**(0.82)**	**bright blue**
Atropine (extracted)		
Solvent system (a)	**(0.75)**	**bright blue**
Solvent system (b)	**(0.80)**	**bright blue**

3. B.4.5.F.T.IR

F.T.IR spectra were measured, Figure (8) shows F.T.IR spectra for atropine that extracted from *datura stramonuim* .The major peaks are listed in table (10)

Table (10) F.T.IR measurement for standard and extracted atropine

Functional group	**Peak value for standard atropine 1\cm**	**Peak value for extracted atropine 1\cm**
C-H aromatic	**3095**	**3020**
C-H aliphatic	**2930-2800**	**2995-2846.7**
O-H	**3500-2700**	**3458.1**
C=O	**1720**	**1717**
C=C	**1600**	**1573.8**
C—O—C	**1245-1110**	**1259.5-1116.7**

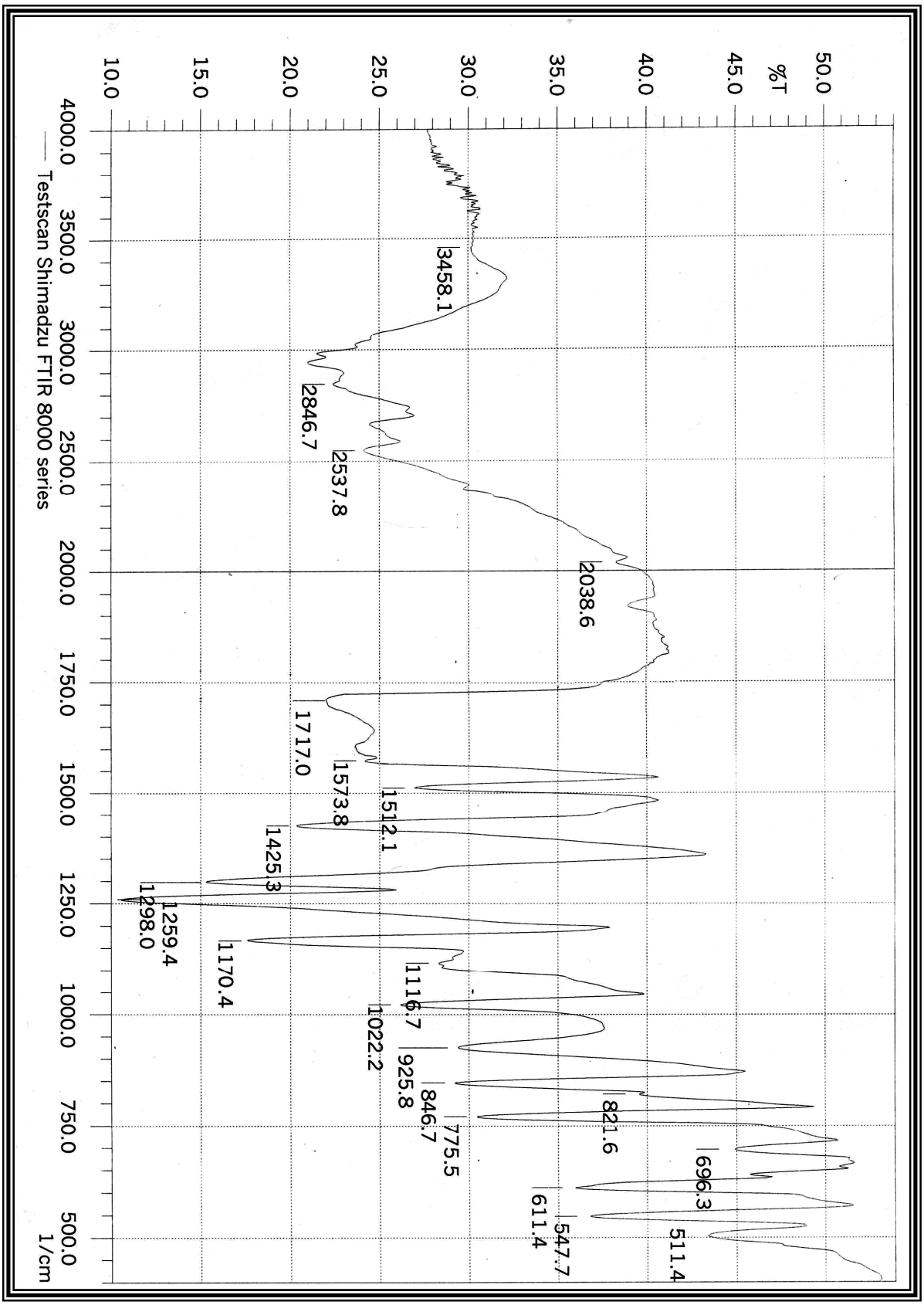


Figure (8) F.T.IR spectra for atropine that extracted from *datura stramonium*

3.b.5.Flavonoids

3. B.5.1H.P.L.C:

The HPLC analysis was done using tetrahydrofuran: deionized water: acetic acid in (35:65:1) v/v as a mobile phase. A 5µg/ml each of standard solutions of different Flavonoids were injected in HPLC. The retention times values of standards qurecitine and rutin were found equal to 13.6 min and 3.45 min, respectively. Analysis of total extract of *Datura stramonium* show retention times equal to 13.59 min and 3.45 min which is comparable to the retentions times for qurecitn and rutin respectively. Figure (7) shows HPLC analysis for total extract of *Datura stramonuim* :

Table (11) Show HPLC analysis for querecitine and rutin

	Retention time(min)
Standard Quercetin	**13.6**
Quercetin in total extract	**13.59**
Standard Rutin	**3.45**
Rutin in total extract	**3. 47**

3.B.5.2. Flavonoids extraction:

Extraction of Flavonoids was done under procedure given in (2.6.) The net weight of crude Flavonoid was found equal to 0.0035g.with 0.035% as a percent.

3.B.5.3. T.L.C

T.L.C measurement was done under procedure given in (2.6) using two solvent system;

a-ethylacetate/formic acid/methylethylketon/water (5:1:3.5:0.5)

b-toluene/chloroform/acetone (40:25:35)

The R_F value for standard solutions of qurecitin and rutin were found equal to 0.4 and 0.35 respectively in solvent system (a). In solvent system (b) the R_f equal to 0.8 and 0.75 respectively. Querecitine, rutin that found in crude flavonoids show R_f value identical in solvent system (a) which equal to 0.4, 0.35. while in solvent system (b) they were equal to 0.83 and 0.73 respectively .

Table (12) R_f values for Qurecitin an Rutin

	R_f		
	Querecitine	**Rutin**	**Color in UV radiation**
(standard)			
Solvent system(a)]	(0.4)	(0.35)	bright yellow
Solvent system (b)	(0.8)	(0.75)	bright yellow
(in crude flavonoid)			
Solvent system (a)	(0.4)	(0.35)	bright yellow
Solvent system (b)	(0.83)	(0.73)	bright yellow

Antibacterial activity;

The antibacterial activity of the water extracts of *Datura stramonium* and *Atropa belladonna* was determined The zone of inhibition of bacterial growth were measured in mm depending upon the diameter as shown in table (13) in *Atropa belladonna* each the four concentration shows biological activity in *E.coli* with different inhibitions zone, but in *Staph aureaus* 5mg/ml and 10mg/ml dose not give any biological activity. In *Datura stramonium* all the four concentrations gave +ve result with different inhibition zones this difference appeared from the alkaloids contents of each plant that occur in different concentrations.

Table (13) Shows Antibacterial activity of *Atropa belladonna* extract

Concentration	*Staph.aureaus*		*E.coli*	
	Result	inhibition zone	Result	inhibition zone
5mg/ml	-ve	-	+ve	7mm
10mg/ml	-ve	-	+ve	8 mm
15mg/ml	+ve	20mm	+ve	11mm
20mg/ml	+ve	21mm	+ve	17mm

Table (14) Show Antibacterial activity of *Datura stramonium* extract

Concentration	*Staph.aureaus*		*E.coli*	
	Result	inhibition zone	Result	inhibition zone
5mg/ml	+ve	5mm	+ve	11mm
10mg/ml	+ve	8mm	+ve	11mm
15mg/ml	+ve	11mm	+ve	12mm
20mg/ml	+ve	10mm	+ve	19mm

Figure (9) show the biological activity against for the two aqeous extract in *E.coli* bacteria

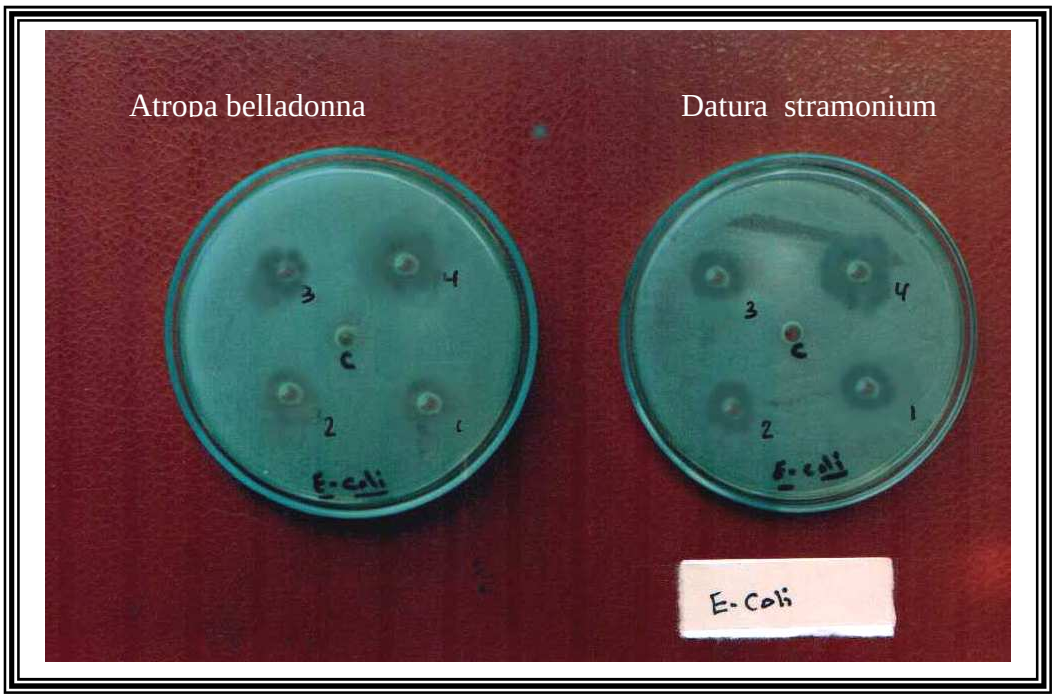


Figure (10) show the biological activity against for the two aqeous extract in *Staph.aureus* bacteria

Conclusions

1. It is possible to extract atropine as one of important alkaloids compounds from *Datura stramonium* and *Atropa belladonna* using methanol as a solvent of extraction.

2. The presence of a new flavonoids compoundwhich is Rutin and Qurecetine in *Datura stramonium* and Catechin in *Atropa belladonna*.

3.Also detection of different biologically active compound in crude plants.

4.Also appearance of a biological activity of the aqeous extract against gram+ve and gram-ve bacteria.

Future work

1. Extract and isolate and identification atropine and other constituents from Atropa belladonna, Datura stramonium.

2. Extract and isolate atropine compound from other plants of solanacea *family.*

3. Study the biological activity for polar and nonpolar extract for atropa belladonna *and* datura stramonium *in solvents such as ethanol, methanol, ether, and hexane.*

4. Study antitumor, anticancer and antifungal activity for polar and non polar extracts.

1.1. Medicinal plants

Humans have always experimented with substances derived from minerals, plants, and animal parts to treat pain, illness, and restore health. In ancient Egypt, physicians prescribed figs, dates, and castor oil as laxatives and used tannic acid to treat burns. The early Chinese and Greek pharmacies included opium, known for its pain-relieving qualities, while Hindus used the cannabis and henbane plants as anesthetics and the root of the plant *Rauwolfia serpentina*, which contains reserpin as a tranquilizer. A school of pharmacy established in Arabia from 750 to 1258 AD discovered many substances effective against illness, such as burned sponge (which contains iodine) for the treatment of goiters anon cancerous enlargement of the thyroid gland, visible as a swelling at the front of the neck[(1)]. In Europe, the 15th century, Swiss physician and chemist 'Philippus Aureolus' Paracelsus identified the characteristics of numerous diseases such as syphilis, a chronic infectious disease usually transmitted in sexual intercourse, and used ingredients such as sulfur and mercury compounds to counter the diseases. During the 17th and 18th centuries, physicians treated malaria, a disease transmitted by the bite of an infected mosquito, with the bark of the cinchona tree (which contains quinine). Heart failure was treated with the leaves of the *Foxglove* plant (which contains digitalis); Scurvy, a disease caused by vitamin C deficiency, was treated with citrus fruit; and small-pox was prevented using inoculations of cells infected with a similar viral disease known as cowpox. The therapy developed for small-pox stimulated the body's immune system, which defends against disease-causing agents, to produce cowpox- and smallpox-specific antibodies. In the 19th century scientists continued to discover new drugs including either, morphine, and a vaccine for rabies, an infectious, often fatal, viral disease of

mammals that attacks the central nervous system and is transmitted by the bite of infected animals. These substances, however, were limited to those occurring naturally in plants, minerals, and animals [2]. A growing understanding of chemistry soon changed the way drugs were developed. Heroin and aspirin, two of the first synthetic drugs created from other elements or compounds using chemical reactions, were produced in the late 1800s. This development, combined with the establishment of a new discipline called pharmacology, the study of drugs and their actions on the body, signaled the birth of the modern drug industry[3]. Extract of many medical plants are used for treatment of many diseases such as proteins that extracted from *Datura stramonium* for inhibiting of Tobacco Mosaic Virus (TMV) [5] and use of *Hyoscyamus niger* plant for treatment of some eyes diseases [2], also use of *Areca Catechu* plant extract for veterinary purpose. There are many studies on the extract of some medical plants for bacterial resistance such as *Bacillus subtilis*, *E.coli*, *Salmonella typhus* [6] and fungi resistance such as *Cladosporium cucumerium* [7, 8], and for Parasites resistance such as *Plasmodium falciparum*. [9, 10]

1.2. Herbs preparation for use:

Herb medicine is usually administrated in the form of tea powdered or as pulps powder's which was sufficiently found to enable the digestive juices to extract the active principles. An administration in powder form allows the body to absorb these principles progressively and totally it will be correct to regard this as the best method of usage. In general the requisite amount of powder drug is suspended in a little water and then swelled vegetable pulps are must often applied to wound. It is necessary to wash the plant or part of plant well, to place it on a clean surface and then bruise it with a knife. The procedure is liable to introduce leaving microorganism into wound. [11,4]

The powdered herb can be prepared by either infusion or decoction. In fusion consist of pouring boiling water on a suitable amount of drug and then allowed to stand. Decoction consists of placing the drug in cold water and then raising it to boil and then allowed to stand, many active constituents are altering by this process of decoction. The fineness of the powder is of a great importance in most cases, the active principles are enclosed within the vegetable cells from which they must be extracted by infusion or decoction. Thus it is necessary to grind bark, root and wood or thick leaves such as bearberry into very small fragments before making teas from them. If this is not done then will be a danger that only a small part of the active constituent will pass into the solution. The amount of drug to be used depends both the patient to be treated and on the nature of the drug used[11]. In the descriptions of the plants the quantities to be used have always been indicated, but these should be reduced for weak Patients. [12]

1.3. Active constituents in Herbs

In order to understand the uses of medicinal plants and their mode of action, it is necessary to know something about their active constituents and their effectiveness of these. Plants are very variable in amount they may even be entirely absent if, for example, the plant has been grown under very unfavorable conditions or if the plant has a low or race active constituent .On the other hand the amount of active principles at some times may be higher than normal and under such circumstances the plant will have a stronger action. For this reason, the pharmacist has to provide the physician and patient with drugs of good average equality.[13]. Modern researches have shown that the action of medicinal plants is due to relatively small number of constituent called "active principles" produced by a plant:

1.3.1Mineral constituent;

Among the mineral constituents of the body, .the salts of potassium and calcium are especially important. Potassium salts poses diuretic properties[14] . While calcium salt contributes to bone structure, to the regulation of the nervous system and to resistance of patient to infection. Organic acids (malic, citric, tartaric, oxalic, etc) are also common constituent of plant they accumulate for example, in fruit. They act in certain cases as mild laxatives, especially tartaric acid and its salts [5] .

1.3.2. Mucilage;

They have in the plant property of swelling in water to produce plastic masses or viscous solutions. It is this property that produces their laxative effect. [14]

1.3.3. Glycosides ;

They are substances that are decomposed into a non-sugar part and one or several sugars when hydrolyzed by enzymes, dilute acids or alkalis or by boiling. Their medicinal action is due to the non-sugar part of the molecules, which are chemically very diverse. [14] The sugar part of the molecules generally influences the solubility in water, and hence it's absorption by the blood. [14,15]

1.3.4. Saponins;

They are also glycosides their outstanding physical character is that their aqueous solutions froth greatly. This is the reason for their use as detergents and it is explain their name (sapo, in Latin mean soap). Large doses in the blood are dangerous and may prove fatal by dissolving the red blood corpuscles (haemolysis)[16] . They are only feebly absorbed from the gastro intestinal tract, their administration by mouth is generally without danger. In the intestine they facilitate the resorption of certain substances (foods or medicines)[17,18]. Like all other glycosides they are destroyed and loose their activity if their aqueous solutions are boiled. [19]

1.3.5.Tannins ;

They have property of precipitating proteins for this reason they convert animal skins in-to leather.[20,21] Tannins prevent the development of bacteria, since the proteins necessary for their nutrition are removed and also their own protein contents are precipitated.[23,21]

1.3.6. Volatile oils or Essences ;

`They are among most utilized product in popular medicine. They are very volatile especially in steam[24]. Their presence is the principal cause of characteristic plant odors [22]. Their medicinal activity is very volatile. Some act on the central nervous system e.g., (anise oil), other increases the secretion of gastric juices (saliva, stomach and intestinal juices) and hence increases appetite. [24,22]

1.3.7. Resins;

They are secreted by special glands similar to those that produce volatile oils and frequently at the same time as these. They are not volatile; they are used as skin irritants. [13]

1.3.8. Bitter principles;

They are groups of substances give bitter taste to the plants. [20]

1.3.9. Antibiotics;

They are extracted from the so-called lower plants (of which the best known is penicillin) [25] are of the utmost medicinal importance since they cure a number of infectious diseases. [19]

1.3.10. Alkaloids;

Alkaloids are naturally occurring compounds having a basic character and containing at least one nitrogen atom in heterocyclic ring[26]. Alkaloid is usually colorless, crystalline, nonvolatile, insoluble in water but soluble in organic solvents some of alkaloids are liquids, optically active and they are generally territory nitrogen compounds. The source of alkaloids may be from animal or plants.[27]

1.3.10.1. Alkaloid of plants:

Typical alkaloids of plants are the nightshade plants (*Solanaceen*). [24] In addition belong the *Black bilsenkraut* (*Hyoscyamus the niger* l., alkaloids: scopolamine, hyoscyamin,). [28] The common *Stechalpfel* (*Datura stramonium*, atropin, hyoscyamin). The *black toll kirsche* (*Atropa belladona,* hyoscyamin and atropine and traces of scopolamin). The *alraun* (*Mandragorum officinarum,* scopolamin, hyoscamine, mandragorin). *Stechapfel*; more scopolamine than atropine. *Tollkirsche* exactly in reverse. Another typical alkaloids of plants are the *Sleep poppy* (*Somiferum*; morphin, codin, , papaverin, narvein).[29] The alkaloids own from the *Sleep poppy* work pain satisfying even in cases in which other means fail. In addition they possess cough-satisfying and cramp-solving characteristics. Also the ergot alkaloids find versatile and mental disorders .one of the other medically more important alkaloid plants are still the white germer (*Veratrum album*), the *herbstzeitlose* (*Colchicm autumnale*),[30] The blue iron herb (*Aconitum napellus*), and the large *Schoellkraut* (*Chelidonium majus*) to be mentioned.[3]

1.3.10.2. Classification of alkaloids:

There is no class of naturally occurring organic substances shows such an enormous range of structures as the alkaloids. This classification occurs according to the location of the nitrogen atom in certain structural features and subdivided into types with distinct chromofor or fundamental skeleton,

1-Alkaloids with exocyclicnitrogen atom.,Inuddenine-12-one compound [1] (30,3)

2-Terpen alkaloids veatchine skeleton compound [2] (30)

3-Steroidal alkaloids compound [3] (32)

4-Hetrocyclic alkaloids: the following group is the backbone of this type of alkaloids (30)

A-Pyrrolidine alkaloids: consist of pyrrolidine group compound [4] (3)

B-Indole alkaloids: consist of indole group compound [5] (27)

C-Piperidine alkaloids: consist of piperdine group compound [6] (3)

D-Pyridine alkaloids: consist of pyridine group compound [7] (33)

E-Isoquinoline alkaloids: consist of isoquinoline group compound [8] (3,30)

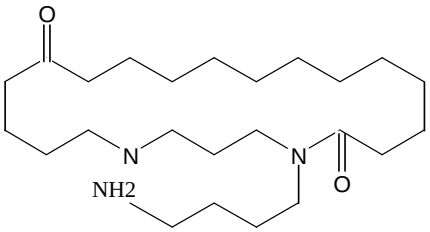
F-Quinoline alkaloids: consist of quinoline group compound [9] (30)

G-Acridine alkaloids: consist of acridine group compound [10]

H-Quinazoline alkaloids: consist of quinazoline group compound [11] (1)

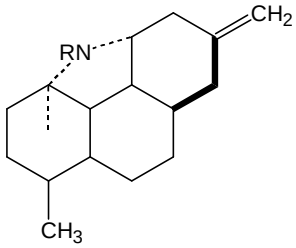
I-Izidine alkaloids: consist of izidine group compound [12] (34)

J-Tropane alkaloids: consist of tropane group compound [13] (34,30)



Inuddenine-12-one

1



Veatchine skeletone

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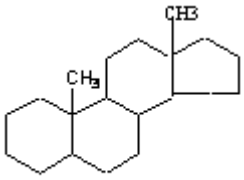

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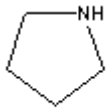

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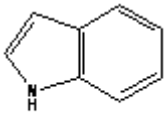

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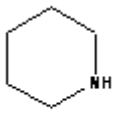

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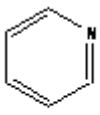

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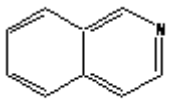

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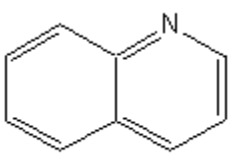

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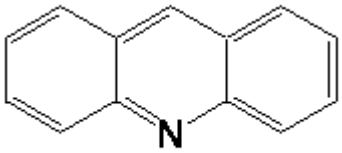

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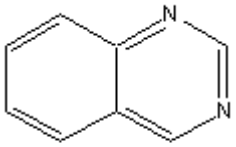

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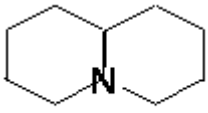

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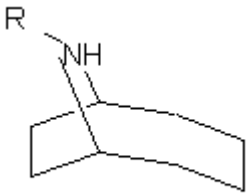


13

1.3.10.3. Tropan alkaloids:

This type of alkaloids is produced by plant of family *Solanacea* such as *Hyoscymus niger*, thorn apple and deadly nightshade.[35] Although the tropan alkaloids were not the first ones detected in nature they early attracted serious chemical and pharmaceutical interest.

Such group of alkaloids is those possing the tropan nucleus [13]. [36]

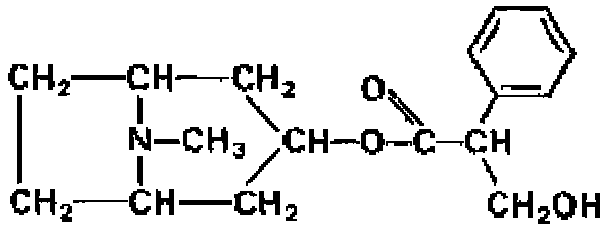
This type of alkaloids is generally esters of tropan-3-ol or related bases. Tropine was known to be saturated and contain a tertiary N-CH3 (methylation yields quaternary salt) and the tropane alkaloids are based

on a 1,4-nitrogen bridged cycloheptane the quaternary salt contain only one more carbon and two HOFMAN degradation yield amine and secondary hydroxyl since it could be reversibly oxidized to aketo, tropinone. The hydroxy is the site of the tropic acid esterfication in the natural alkaloids.[37] The most important compounds of alkaloids are (-) – hyoscyamine,[39] scopolamine and hyoscine.[38]

1.3.10.4. Atropine: -

Atropine [14] is an anti-cholinergic drug and it is not found in natural alkaloids form alone, but rather as a racemic mixture with hyoscyamine.[3] The chemical formula of atropine and the structural form is

$C_{17}H_{23}NO_3$



[14]

Molecular weight: 289.38 There are other names of atropine, atropine-Flexiolen, atropinol, atropisol, Alpha- (hydrozymethyl)-18-methyl-8-azobicyclo (3,2,1) oct-3-yl-esterendo (±); Eyesules; endo- (±)-alpha-(hydroxymethyl) benzene acetic acid-8-methyl-8-azabicyclo [3,2,1] oct-3-yl ester;[40,41] *d*-Hyoscyamine, *dl*-Hyoscyamine, isopto-atropine;beta-phenyl-gamma-oxypropionsaure-Tropyl-ester, (German). 1-alpha,5-alpha-tropan-3-alpha-ol(-+)-tropate(ester);*dl*-tropanylz-hydroxy-1-phenylpropionate;tropic acid ester with tropine; tropane tropate; tropin tropate (ester); DL-tropyl tropate; (±)-tropyl tropate.[42]

1.3.10.4.1. Properties:

Atropine appear in long, orthorhombic prisms from acetone, and rhombic needles from dilute alcohol this difference due to the crystal formation from different solvents.[43]

Melting point: 114-116^{o} C [44]

Solubility: 1 gram in 455 ml water
2 ml alcohol
25 ml ether
27ml glycerol
1 ml chloroform.

It is colorless crystal or white crystalline powder odorless.[43] When dried at 120 c^{o} for 4 hours it is melts at temperature not below 187^{o}C.[45]

1.3.10.4.2 Action:

Clinically the atropinic drugs cause mydrisis and cycloplegia and can be used locally to induce mydrisis for though eye examination[46]. Atropine is used to reduce salivary and bronchial secretions by smooth muscle relaxation of bronchi.[47,48] The principal effect of atropine on the heart is to alter rate. At low doses the rate is slowed without a change in blood pressure or cardiac out put, large doses cause an increase heart rate. Atropine may be used in initial treatment of myocardial infarction or high-grade atroventricular block.[49] The tropan alkaloids are also used in peptic ulcer therapy because they decrease gastric secretion and total acids content in moderate doses.[50,51] Clinical doses of atropine cause mild excitation of the central nervous system[52]. At steadily increasing doses central excitation is increased but then central depression follows. Atropine is also useful in cases of poising. In large doses atropine may also be used in the treatment of anticholinesterase poisoning by organophrous insecticides.[53,54] .

Atropine can also be used for the treatment of the galena or kidney colic. Because it is restrain the vagus, is to be expected that their application lead to reduction of the pain-realizing, contractions of the smooth musculature. Atropine combats the effect of the nerve agent in the airways. Atropine reduces secretions and relieves the narrowing of the airways that otherwise result in chocking to death.[55] The average dose of atropine was 0.5 mg, the effect appears toxic in most cases it exceed 10 mg or more.[21] The side effect associated with use of atropine rare unsteadiness, hallucinations, unusual dryness of mouth and

increased sensitivity of eyes to light.[45] Patient who has glaucoma should be use atropine with caution.[42] .

1.3.11. Flavonoids

Flavonoid consisted one from of the most numerous and widespread group of naturally occurring oxygen heterocyclic compounds. They occur usually as glycosides in all types of higher plant tissue-wood, bark, stems, leaves, fruits, roots, flowers, pollens, and seeds, but they are of restricted distribution in lower plants [56]. Certain groups of Flavonoids are more characteristic of some tissues than others. In fruit bearing plants, however, the same groups of flavonoid that occur in the leaves as well as the fruit in a lesser amount.[57] Flavonoid contains a 1,3-diarylpropan carbon skeleton which is accommodated within a 2-phenyl chroman structure.[58]

The yellow, red, blue and violet pigments found in various parts of plants are regarded mostly as one or more of the different types of flavonoids.[59]

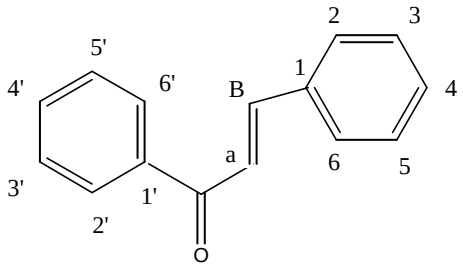
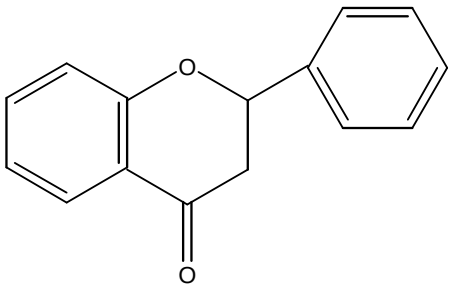
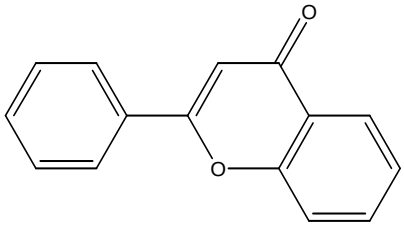
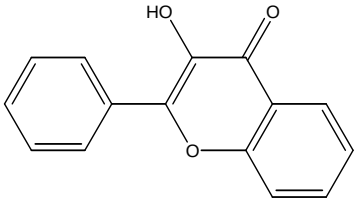
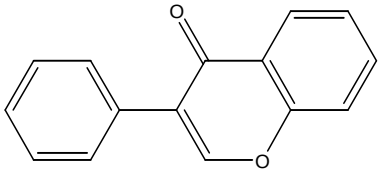
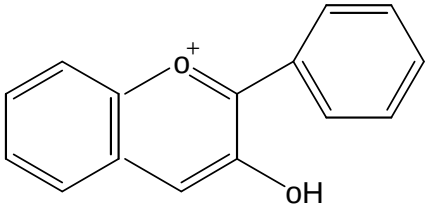
1.3.11.1. Classification and distribution in nature:

The aglycone part of flavonoids is classified into several groups according to the oxidation levels which are possible in the heterocyclic ring. Each group consists of several examples resulting from the differences in the oxygenation as well as substitution pattern in the two aromatic rings.[60]

The major flavonoids of the plants are the anthocyanins [19], flavones [16] and flavonols [17] which are accompanied in certain plant groups by the minor flavonoids including the flavanones [15], isoflavones [18], chalcones [14]. Although the latest two groups do not fall into the general pattern of

flavovoids, they are related biogenetically to them and are thus included in flavonoid group.[61] In the plant kingdom the flavonoids are distributed in a variable proportion from one class to another. The angiosperms are regarded as the biggest class, it accounts for between 250-300 thousand species are cultivated or gathered as human food. Flavonoids and related compounds have been isolated from or detected in about one-half of these edible plants, but not always in the edible portions.[62,63]

The gymnosperms are considered relatively a small group, and it has been extensively surveyed for flavonoids.[57] The main chemical feature distinguishing the gymnosperms from both lower plants and the angiospermsis bioflavonyl formation.[58] Biflavonyls based on amentoflavone, formed from two molecule of apigenin by carbon-carbon coupling at the 8 and 3'-positions, are widely distributed in gymenosperms especially in two families, the *Pinsceae* and the *Ephedraceae.* The *Thallophyte,* a class of lower plants, is characterized by the limited distribution of flavonoid in most of the plant orders of this group.[57] Flavonoids classes and basic structure shown below:

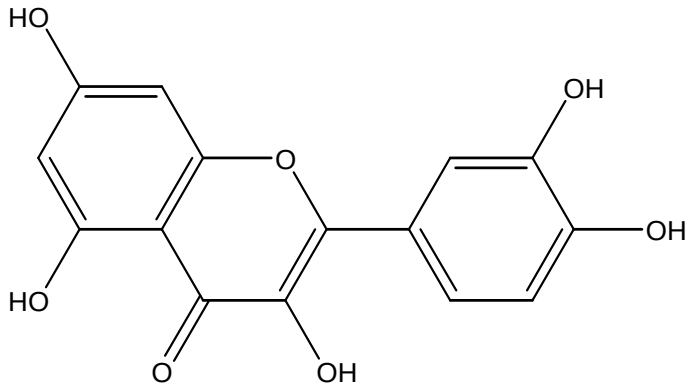
	Flavonoid Class	Basic structure
1	Chalcones	[14] 
2	Flavanones	[15] 
3	flavone	[16] 
4	flavonol	[17] 
5	isoflavone	[18] 
6	Anthocyanins	[19] 

1.3.11.2. Flavonols:-

Flavonols [17] can be regarded as 3-hydroxyflavones; individual differences arise from the number and distribution of the hydroxyl groups as well as from the nature and extent of alkylations and/or glycosylation of these groups. In common with other flavonoids the flavonols [17] most frequently found in plants are those with a hydroxyl group in the 4' position only .(28)

1.3.11.4.1. Quercetin:-

Quercetinis compound [20] a Flavonol that serves as the backbone for many other flavonoids. It is consistently the most active of the flavanoids in experimental studies, and many medicinal plants owe much of their activity to their high querecetin content.(63)



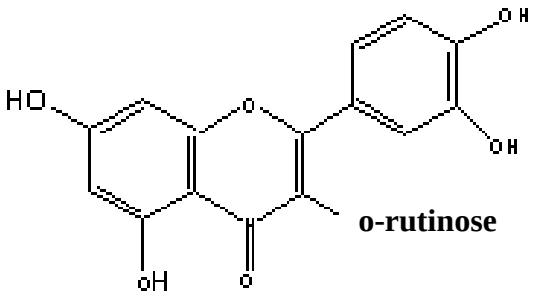
[20] quercetin

Action:-

It is best known as an anti-inflammatory/anti-allergy agent. Because it stabilizes most cell membranes and prevents the release of histamine and other inflammatory agents, it is often prescribed for food and inhalant allergies, asthma, excema, psoriasis, gout, and ulcerative colitis.(57) Due to it is antioxidant effect, querecetin [7] can inhibit inflammatory processes mediated by "leukotrienes" (inflammatory agents a thousand times more powerful than histamines).(56) Quercetin(19) can significantly decrease the accumulation of "sorbitol" in the lens of diabetic animals, effectively delaying for it's ability to enhance insulin secretion, protect the pancreatic beta-cells from the damaging effects of free radicals, and inhibit platelet aggregation.(59)

1.3.11.4.2 Rutin:-

The Flavonoid Rutin [21] found in many plants, especially the black wheat plant *Fagopyrum esculentum*, the flower of which is used to make panacakes. Other rich dietary source of Rutin [21] include black tea and apple peels.(28) It is solid substance, pale yellow in appearance and only slightly soluble in water. Rutin has molecular weight of 610.53 with the molecular formula $C_{27}H_{30}O_{16}$ its structural formula is shown (56) bellow



o-rutinose

[21]

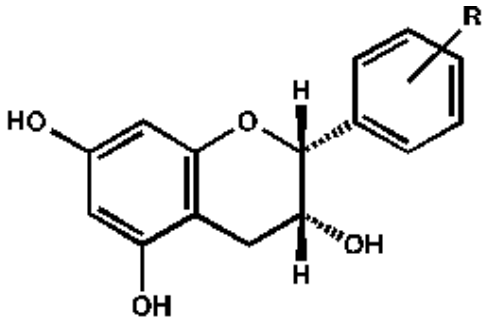
Action:-

Rutin [21] may have antioxidant, anti-inflammatory, anticarcinogenic, antithrombotic, cytoprotective and vasoprotective activities. It may be useful in the management of venous edema.(64) It may help strengthen capillaries, protect against some toxins and have anti-inflammatory effects, as well as some anticancer effects(61). It may also help prevent the oxidation of vitamin C and have some positive lipids effects.(61)

1.3.11.4.3 Catechine

Catechins [22] belong to the flavan-3-ol class of Flavonoids. It is white powder have molecular formula $C_{15}H_{14}O_6$ and it is molecular weight equal to 290**.** 3

[22]



Action:-

Have antioxidant activity, they may also have anticarcinogenic, anti-inflammatory, anti-atherogenic, thermogenic and antimicrobial activities(64).

1.4. Active constituent extraction:

Ideally, fresh plant tissue should be used for photochemical analysis and the material should be plunged into boiling alcohol with minutes of collection. Sometimes freshly picked tissues, stored dry in a plastic bag, will usually remain in a good condition for analysis. (65)

Alternatively, plants may be dried before extraction. If this done, it is essential that the drying operation is carried out under controlled conditions to avoid too many chemical changes occurring. It should be dried as quickly as possible, without using high temperature, preferably in a good air draft(66). Once thoroughly dried, plants can be stored for long periods of time. Indeed, analyses for flavonoids, alkaloids, quinones and terpenoids have been successfully carried out on herbarium plant tissue many years ago (67).

The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on the type of substance that being isolated.. Alcohol, in any case is a good all-purpose solvent for preliminary extraction. Subsequently, the material can be macerated in a blender and filtered but this is only really necessary if exhaustive extraction is being attempted.(68) When isolating substances from green tissue, the success of extraction with alcohol is directly related to the extent chlorophyll is removed into the solvent and when the tissue debris, on repeated extraction, is commonly free of green color, it can be assumed that all the low molecular weight compounds have been extracted.(69)

The classical chemical procedure for obtaining organic constituents from the dried plant tissue (heartwood, dried seeds, root, and

leaf) is to continuously extract powder material in Sohxlat apparatus with a rang of solvents, starting with ether, petroleum, and chloroform(to separate lipids and terpenoids) and then using alcohol and ethyl acetate (for more polar compounds). This method is useful when working on the gram scale. However, one rarely achieves complete separation of constituent of the same compounds may be recovered (in varying proportions) in several fractions.[70,71,72]

There are many research that done on the extract of alkaloids from many types of plants, e.g, extraction of nicotine from tobacco[73,74] is an example for extraction of alkaloids using methanol as solvent for extraction. *Solanine* extracted from potato tissue [76] the extraction procedure consist the following :

1. Extract the tissue with 5% acetic acid (15-20 parts) and filter the extract to remove cellular debris. Warm to 70^{o}C and add conc. NH_3 solution drop wise till the pH reaching 10.
2. Centrifuge and discard the supplemental. Wash the precipitate with 1% NH_3 solution and re-centrifuge.
3. Collect, dry and weigh.

Piperine also extracted from *pepper;* [77] the extraction done by using ethanol as a solvent for extraction. Atropine extracted from *Datura innoxia* [78] using 95% Ethanol as a solvent of extraction and extracted by soxlet. Purification of atropine was done using preparative TLC (thin layer chromatography) with 70:20:10 (Ethanol, water, acetic acid) as eluent. Atropine extracted by using water as a solvent of extraction from *Datura stramonum*[79]. The same procedure for extraction of atropine, from *Atropa belladonna* [77] using methanol as a solvent for extraction. Extraction of tropane alkaloid is done by using alcoholic 5% H_2SO_4 as a solvent for extraction [81].

The extract obtained is clarified by filtration through calcite on a water pump and is then concentrated in vacuum. This is now usually carried out in rotatory evaporator, which will concentrate bulky solutions down to small volumes, without bumping, at temperatures between 30 and 40 C^0. The concentrated extract may deposit crystals on standing. If a single substance is present, the crystals can be purified by re crystallization and then the material is available for further analyses. In most cases, mixture of substances will be present in the crystals and then be necessary to re dissolve them up in a suitable solvent and separate the constituent.

Flavonoids extraction depend mainly on the type of Flavonoid being isolated. Solvent used for extraction is chosen according to the polarity of the flavonoids being studied. The less polar solvents are particularly useful for the extraction of flavonoids aglycones, whilst the more polar solvents are used if flavonoid aglycosides or anthocyanins are sought. The less polar aglycones, such as isoflavonoids, flavanones, and dihydro flavonols or flavones and flavonols, which are highly methylated, are usually extracted with solvents such as benzene, chloroform, ether, or ethyl acetate [81]. A pre-extraction with light petroleum or hexane is frequently carried out to get rid of plant material such as sterols, carotenods, chlorophyll [82,83].

Flavonoids glycoside and more polar aglycones such as hydroxylated flavones, flavonols, bioflavonyls, and chalcones are generally isolated from plant material by extraction with acetone, alcohol, water or a combination of these[84].

Perhaps the most useful solvent for the extraction of this group of compounds is 1:1 water:methanol solution .Trace of acid are

occasionally incorporated in the solvent for the extraction of flavonoid aglycosides [85], although this practice is normally re-sieved for the extraction of anthocyanin [81].

1.5. Methods of identification:

Once it has been isolated and purified, it is necessary first to determine the class of compound and then to find out which particular substances it is within that class. The homogeneity must be checked carefully beforehand, i.e. it should travel as single spot in several TLC or PC systems.[70,72] Complete identification within the class depend on measuring other properties and comparing these data with those in the literature. These properties include melting point for solid, or boiling point for liquids, equally informative data on a plant substance are its spectral characteristics these include ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) and mass spectral (MS) measurements.[75] A known plant compound can usually be identified on the above bases. Direct comparison with authentic material (if available) should be carried out as final conformation if it not available, careful comparison with literature data may be sufficient for its identification. [72]

1.6. *Atropa belladonna*

There are other names used for belladonna such as (*Atropa belladonna*, deadly nightshade, dwal, devil's cherries and devil's herb).

1.6.1. Description: -

Perennial plant, 50-200 cm high, with stout branched and thick root. Leaves up to 20 cm long, elliptical, with acute apex haring. Flower corolla up to 2.5cm long, bell shaped, externally dull violet. Internally dull yellowish-brown with purple veins. The flowers, which appear in June and July, are singly in the axils of the leaves, its color is green to purplish color.

1.6.2. Part uses: - root and leaves.

1.6.3. Constituents: -

leaf and root contain highly poisonous alkaloids mainly hyoscyamine. The medical properties of belladonna depend mainly on the presence of hyoscamine. The total alkaloids present in the root vary between 0.4-0.6% consisting hyoscamine 0.1-0.6%, scopolamine (hyoscine) found in traces for leaf and roots. The proportion of total alkaloids present in the leaves varies from 0.3-0.7% the greater proportion consists of hyoscyamine. The atropine being produced during extraction as in the root.

1.6.4. Usage: -

Atropa belladonna should be administered only under medicinal supervision for the treatment of the nervous diarrhea, also for constipation, the treatment of enuresis, and for eye diseases.

. **1.7. *Datura stramonium***

There are other names used for *Datura stramonium* such as Thorn apple, jimsonweed, and stinkweed.

1.7.1. Description:

Annual plant 30-120 cm high. Stems stout, round branched leaves sometimes greater than 20 cm, petiolate, ovate to triangular, margins broadly sinuate-dentate. Flowers solitary in the axils of the leaves with large, white, funnel-shaped corolla. Fruit: a thorny capsule have 4 valves and containing many black seeds. All part of the plant has a disagreeable and nauseating odor. Flowering occurs in June-September.

1.7.2. Part uses: -dried leaves and seeds.

1.7.3. Constituent: -

Datura stramonium contains of the same alkaloids as belladonna but in different proportions. All part of the plant is poisonous. One half of teaspoon equivalent to 0.1mg of atropine. Scopolamine also extracted from it to make an medicinal drug (Buscopane) which is n-bromidbutyl scopolamine.

1.7.4. Usage: -

Datura stramonium should not be used except under medical supervision, to relieve asthma, the tincture is used as anti-asthmatic cigarettes in which *stramonium* are an ingredient. Commonly consumed in herbal tea. The seeds, leaves and flowers nectar can also be eaten or smoked. Ingestion of *Datura stramonium* can lead to seizures, coma and death; symptoms include dry mucous membranes, and blurred vision.

The effect can occur for 24-48 hours. The usual rout of ingestion is as a tea although ingesting seed or other plant part.

Aims of this work

1. Extract and isolate some alkaloids and Flavonoids from two Iraqi plants (*Datura stramonium*, *Atropa belladonna)* and determining the weight of atropine in each of those plants.

2. Investigate the antibacterial activity of the aqeous extract of the two plants in different concentration against some kind of bacteria.

3. The third aim of this work was to detect the presence of other biologically active compounds.

Chapter one
Introduction

Chapter three Result and discussion

Chapter two Experimantal part

Conclusions

1. The ability to extract atropine as one of important alkaloids compounds from *Datura stramonium* and *Atropa belladonna* using methanol as a solvent of extraction.

2. The presence of a new flavonoids compounds in the two plants with a low concentration which was rutine and qurecetine in *Datura stramonium* and catechin in *Atropa belladonna*.

3. Detection of different biologically active compound in crude plants.

4. Appearance of a biological activity of the aqeous extract against gram+ve and gram-ve bacteria.

Chapter two

Chapter three

List of Tables

List of Figures:

Examining Committee's Certification

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(Member)

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Title: Lecturer
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I herby, certify a bone the decision of the examining committee.

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Future work

1. Extract and isolate and identification atropine and other constituents from *Atropa belladonna*, *Datura stramonium*.
2. Extract and isolate atropine compound from other plants of *solanacea* family.
3. Study the biological activity for polar and nonpolar extract for *atropa belladonna* and *datura stramonium* in solvents such as ethanol, methanol, ether, and hexane.
4. Study antitumor, anticancer and antifungal activity for polar and non polar extracts.

References

1.Al-Rawi, A. "***Poisonous Plant of Iraq*** " .Baghdad univ.2nd edition .pp111-113.1980.

2.Cooper. M. R. and Johnson. A.W**. *"Poisonous Plant, in Britain and their effect on animals***" pp:33-34 (1984).

3- Geoffrey A. Cordell." ***Introduction to alkaloids"*** University of Illinois. (1981).

4-J. B.HAB." ***Phytochemical Methods"*** J.B. Harbome.pp57-59: 1973.

5- http // www. Yahoo/ classification alkaloid

6- Dr. Rita JASPERSEN-SCHIB "***Medical Plants and There uses".*** 1976.

7-Mars ton. A.: Maillard miad Hostettmann. K.. Molluscicidal. "***J.Biochemistry*** "38: 215-223 (1993).

8-Muanza. D.N: Kim. B.W.: Euler. K.L. and Williams." ***Alkaloids*** "pp543-544. (1994).

9-Brandao. M. G. L.: Grand: T.S.M.: Rocha. E.M.M.: Sawyer. D.R. and Krettli, A.V.." ***J. Ethnopharm***," 36: 175-182 (1992).

10-Geller. M.C.: Nkunya< M.H.H.: Mwasumbi. L.B.: Heinrich. M. and Tanner. M. . "***J.Acta. Tropica".*** 56: 65-77. 1994.

11- Olukoya, D.K., Idika, N; Odugbemi**...*" J*. *Acta.Pharma"*** 39: 69-72 (1993).

12-WWW. Yahoo/ medical herb. Tea.

13-Micks. R.H.***"The Essentials of Material Medical Pharmacology and Therapeutics***." London. (1961).

14-Swain. T "***Chemical Plant Taxonomy***." 2nd.edition Academic press. London and New York. pp109-111.(1963).

15- Grahams H. Salomons. 3rd edition. Willy press, (1984), 796-797.

16- Nuutila A, K. Kammiovirta and K.M.O. Caldentey.”***J. Food Chemistry***.” 76, pp519-525 (2002).

17-Bitters. W.P. Murashige, T.:Rangan. T.S. and Nauer E. Virol Unv. Floride press(1972).

18-Doby. G “***Plant Biochemistry.***” John wiley and sons. LTD. London. . pp12-14 (1965).

19-Furuya. T. and Ishii. T).”***J.Japan.Pla***”. 48: 31-417. C.F.(1972).

20-Nickel. L.G.***“ Products in Plant Tissue Culture as a Source of Biochemicals***”. CRC press floride, pp1100- 1102. (1980).

21- Antonio.”***Animal Production Practices and Material”*** Federal organic Food productionpp304-306(1991).

22-Heinrich. M.: Kuhnt. M.:Wright. C.Wirimpler. H. “***J. Acta Pharma***, “ 36: 81-85.(1977).

23- Raphael Ikan. Natural products. (1969). Academic press.

24-Timothy A. Forberger. “***Analytical Branch” .*** (1998).

25-Fox. J.E. “***Physiology of Plant Growth and Development***”. P:85-86 (1969).

26-Kamada. H.: Okamura. N.: Stake. M.: Harada. H., and Shimomura, ***“ Alkaloid Production by Hairy Root Cultures: Atrope belladonna***.” pp239-242. (1986).

27-Stockig. I.J.: Pfitzner. A. and Firl. J. “***Indole Alkaloids from Cell Suspension Cultures***”, 36-39. (1981).

28--WWW. Yahoo/ alkaloids

29 –يوسف منصور تصنيف النباتات البذرية. مطيعة / جامعة بغداد(١٩٨٨)

30--Pacheco. P.: sierra. J.: schmed. H.G.: potter. C.W.:Jones. B.M. and Moshref. M. “***J. Phytoth. Es***.” 7: 415-418. (1993)..

31-Mantell. S.H. and Smith. H. "***Plant Biotechnology***". Cambridge. Unv. press. Cambridge. U.K.pp299-305 (1984).

32-Fowler. M.W. "***Plants"***.pp:2-6, (1987).

"***Plant Cell Biotechnology to Produce Desirable Substances. Chemistry Industrial"***, C.F. Mantell and smith pp:229-233. (1984).

33-Ehmke. A. and Eilert. U." ***Plant Cell Report***". 5: 31-34 (1986)

34-Petri. G... "***J.Acta Pharmacy***", 306: 72-82. (1992)

35- Tabata, N. Hiraoka, and M. Konoshima,"***J. Acta Pharma "***, 24:65, 1979

36- Herouart. D. ;Gontier, E.; Sangwan. R.S. and Sangwan., N.B.S. ***"J.Bicche***" 42: 1073- 1076. (1991).

37- Robins. R.J.; Woolley. J. G.; Ansarin. M.; Eagles, J. and Good follow. B.J.., "***J.Planta***", 194: 86-94. (1994)

38- Maysoon.W.S.Omer. ";***Synthesis of Nitrogen. Bridged Bicyclic Molecules***." pp654-660 (1978).

39- httb:// www. Chem.bris.ac.uk/motm/atropine/source.htm.

40- Pacheco. P.: sierra. J.: schmed.H.G.:Potter.C.W.:Jones,"***J. Biochemistry"***, 7:415-418. (1978)

41- Srepel " ***Actapharm***" Jugoslav., , 21:14 1971

42-Richard.A.Wagner,, M.D. Ph.D. "***Poisoning Alkaloids"***(1991).

43-Ramirez M, Rivera E, Ereu C:"***Fifteen cases of Atropire Poisoning after Honey Ingestion***" 19-20(1999).

44-Budavari. S., " ***The Merck Index*** ", Merck and co.Inc. , Rathway, U.S.A., 11th ed., (1989).

45-Kitamurai. Y.; Sato, M. and Miura. H "***Differences of Atropine Esterase Activity***" .(1992).

46-Maldonado-Mendoza. I.E.: "***Alkaloids in some Plants***" pp22-25 (1995).

47-Holmes, H.L.,"***Alkaloids***"NYI, 27 (1950).

48-Robinson. R"***J. Chem. Soc***."111, 762 (1917).

49- Donovan. D.G. and M.F. Keogh."***J.Chem. Soc"*** Ser, 223 (1969).

50-Fodor. G., in S.W. Pelletier (Ed)."***Chemistry of the Alkaloids***." Van Nostrand Reinhold. New York. 431, (1970).

51.Christen,P.: Margaret.R.R:Phillipson.D.J and Evans, W.C, "***Plant Cell Report"***.8:76-77.(1989)

52.Saxton.J.E. "***Alkaloids".*** London 5-9 (1979).

53- Botini.A.T.,and J.Gal."***J.Org.Chem"***.36:1718(1971)

54. Parker.W.R.A.Raphael. and D.Wilkinson."***J.Chem.Soc".***24:53 (1959).

55.McGaw.B.A.and J.G.Wooley,"***J. Phytochemistry"***.17:259(1978).

56.Geissman,T.A, (1962), "***The Chemistry of Flavonoids Compounds"***, 1st Ed, Pergaman Press, oxford.

57. Harbon, J.B.,"***General Distribution of Flavonoids Compounds'"***, pp.101-103, academic press.london and New York. (1967).

58.Geissman,T.A.and Crout,D.,H.,G., "***Organic Chemistry of Secondary Plant Metabolism"***, Freeman, Coper and Co.,California.(1969).

59.Claus,P.,E.; Tyler,E.V and Brandy ,R.L,"***Pharmacology",*** pp.120-122, 6th Ed., K.m.,Varghesecompany Bombay.(1970)

60.Harbone,J.B.,"***Hytochemical Methods"*** pp52-55, chapman and Hall, London.(1973).

61.Fratt,E.D., "P***henolic,Sulfer and Nitrogen Compounds in Food"***pp1-13, Charalambous G .,West Lafayette, Ind.(1979).

62.Harone, J.B."***Anthocyanin Pigments"*** pp1-30. Acadimic Press, London and New York.(1967).

63.Angale S.Simman.Baghdad univ.Ph.d***Thesis*** .(1989).

64.Hermannk., "***.Food Tech"***11:433-440 (1976).

65.BRAND,J.C.DandEGLINTON,G."A***plicationofSpectroscopy"***.London.p p234 (1965).

66. Bradford, M.M."***J.Ann.Biochem***"72:248-254.(1976)

67.Williams, D.H.and FLEMing,.I. "***Spectroscopic Methods in Organic Chemistry"***.McGraw Hill Publ.co.london. pp:1222-1230 (1966).

68. SCHEINMAMN,"***An Introduction to Spectroscopic Methods for the Identification of Organic Compounds***" .Oxford.Vol.I.pp201. F.ed.(1970.

69.MABRY, T.J"***In Perspective in Phytochemistry***". Harbone,J.B and swain, T., Academic press. Lond. pp. 1-3 .(1969)

70.Rincon.J.A.;M.A.Garcia;A.Garcia;A.Delucas and A.Carnicor. "J.***Separation Science of Technology"***.Vol 33, Page 411. 1990.

71.Ray Q.;E.W.Brewster,"***Organic Chemistry***" ,Prentice-Hall 3rd , ed.Page.751.1971.

72. Dubois M.:Gilles, K.A.; Hamilton, J.K.; Rebers, R.A.;Smith, ***J.Anal.Chem***.28:350-356. 1956

73. Mrs.M.Grieve."***Amodren Herbal***".2000

74. SCOTT.A.I"***Interpretative of the of the U.V Spectral of Natural product***".Oxford pp(301-305). (1964).

75.Leslie.D.C.;Millen;W.R.Murphy."***Ind.Eng.ChemistryResearch***", 32:12,1993.

76.Saxena,P,K.:Cill.R.:Rashid.A.and Maheshwara.S.C.(1982).

77-Ikan, R "***Natural Product. A.lab. guide***." Acadimic press. pp.178-179, .(1969).

78.Szoke.E.:Dung.N.N.: Petrii.G.V and potoczkis".***J.Acta Pharma"***.29: 403-410.(1982).

79.Ballica.R.; Ryu.D.D.Y and Kado .C.I. ***Biotechnol.Bioeng***.11:1075-1081. .(1993)

80.Fahmy, I.RConstituents of plants and crude drugs.1st ed .cairo. .(1933)

82. Flukie, H. ***"Planta Media***". 61:138-140. (1995)

83. Ducke, J.A "***Hand Book of Medicinal Plants"***. Florida .(1985).

84. Dubois, M. "***Anal .Chem***". 52:350-356 . (1956).

85. Al-Kaise, M.T "***Arbic.Sci***". 5 (1):21-28

References

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Extraction of Medicinal constituents from two Iraqi Herbs *Datura stramonium* and *Atropa belladonna* -A chemical study-

A Thesis

Submitted to the College of Science Al-Nahrain University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

By

Bassam Faron Abed Al-Farhany

(B.Sc. 2002)

May 2005

Rabee Al Thany 1426

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم الكيمياء

استخلاص مكونات طبية من عشبتين عراقيتين الداتورة و ست الحسن
- دراسة كيميائية-

رسالة
مقدمة الى كلية العلوم- جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

من قبل
بسام فرعون عبد الفرحاني
بكالوريوس ٢٠٠٢ (جامعة النهرين)

ايار ٢٠٠٥ **ربيع الثاني ١٤٢٦**

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الديوانية
ز١٧٣ .م١١٥ . د٩

ألاهداء

الى نبي الرحمة و اله

قاصدا شفاعتهم

الى من احال ثمار الكدح المرة على شفاهنا عسلا دون دموع

الى من اسمائهم محفورة في قلبي.....ابي و امي

طمعا برضاهم عني

الى نسمات الامل الى اخوتي.....

احتراماً لما تحملوه من أجلي

الى كل قلب خفق حباً لي وخوفا عليَ

أهدي هذا الجهد المتواضع مع خالص حبي وامتناني

بسام فرعون

2.1 Standard solutions:

The following standards were obtained from different company as listed below

Compound name	Source	Type
Quercetin	**Fluka**	**Flavonoid**
Catchin	**Fluka**	**Flavonoid**
Rutin	**Fluka**	**Flavonoid**
Atropine	**Sigma**	**alkaloid**

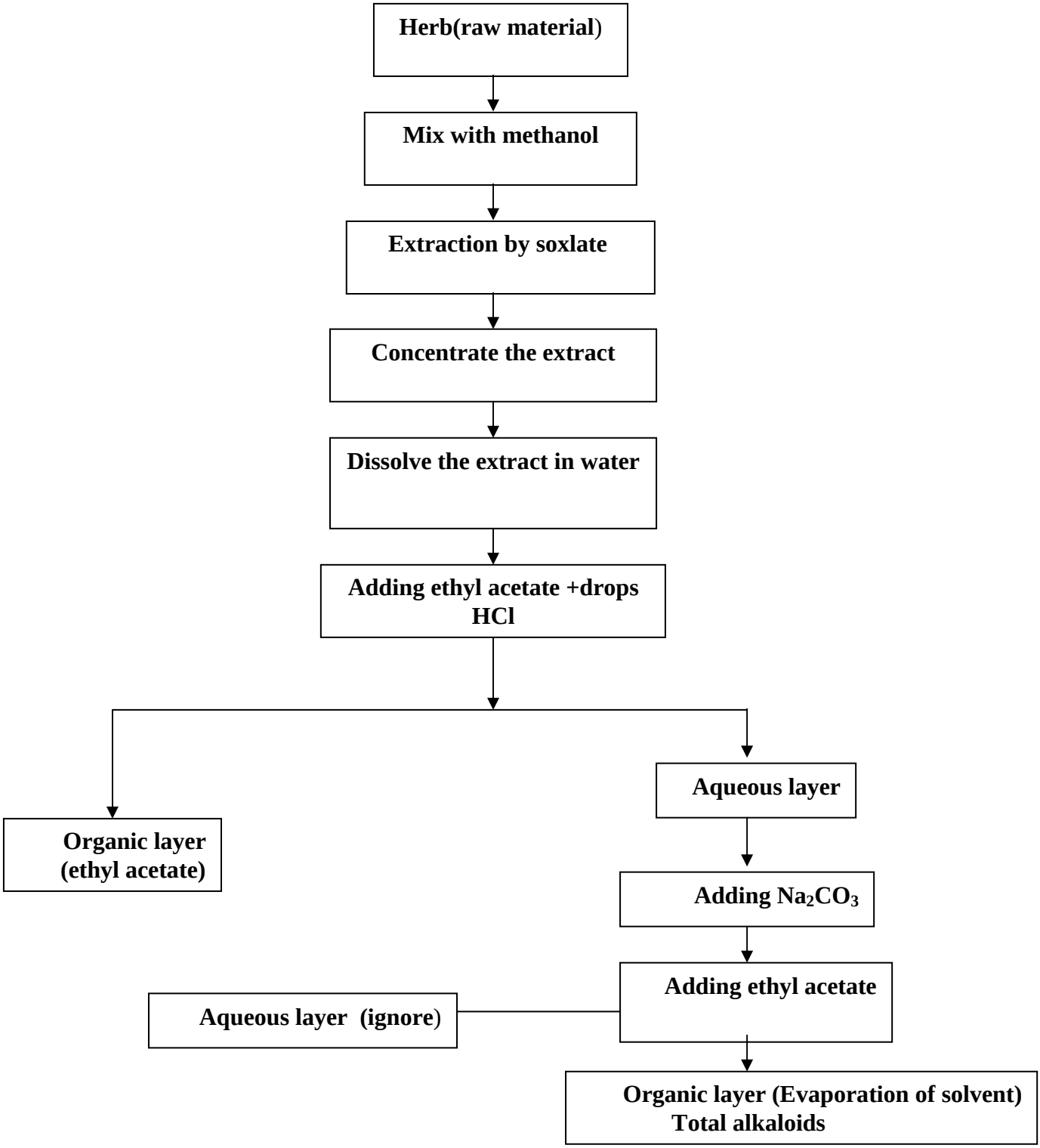
2.2. Preparation the Plant for extraction:

Samples of Iraqi plants (*Datura* and *Belladonna*) were taken from local market and home gardens identified in Herbal Medicine Center by Dr. Anan. Sample were washed with cold water to remove any dust and then dried in oven at 50^0 C for 3 hours, then the samples were grinded to a fine powder and used for extraction.

2.3. Alkaloids extraction

A 10 gram sample from the plant sample was taken and mixed with 50 ml methanol and extracted by soxhlet for 6 hour. The extract was concentrated using water bath after that the thick extract dissolved in 25 ml water. 25 ml ethyl acetate was added to the solution and acidified using few drops of HCl. The separation of resulted two layers was done using separatory funnel. The aqueous layer was taken and 25 ml Ethyl acetate was added and neutralization using sodium carbonate solution and then evaporated the organic layer (ethyl acetate) to get alkaloid.[63]

The scheme (1) bellow shows the extraction procedure:



Scheme (1) Extraction procedure for alkaloids [99]

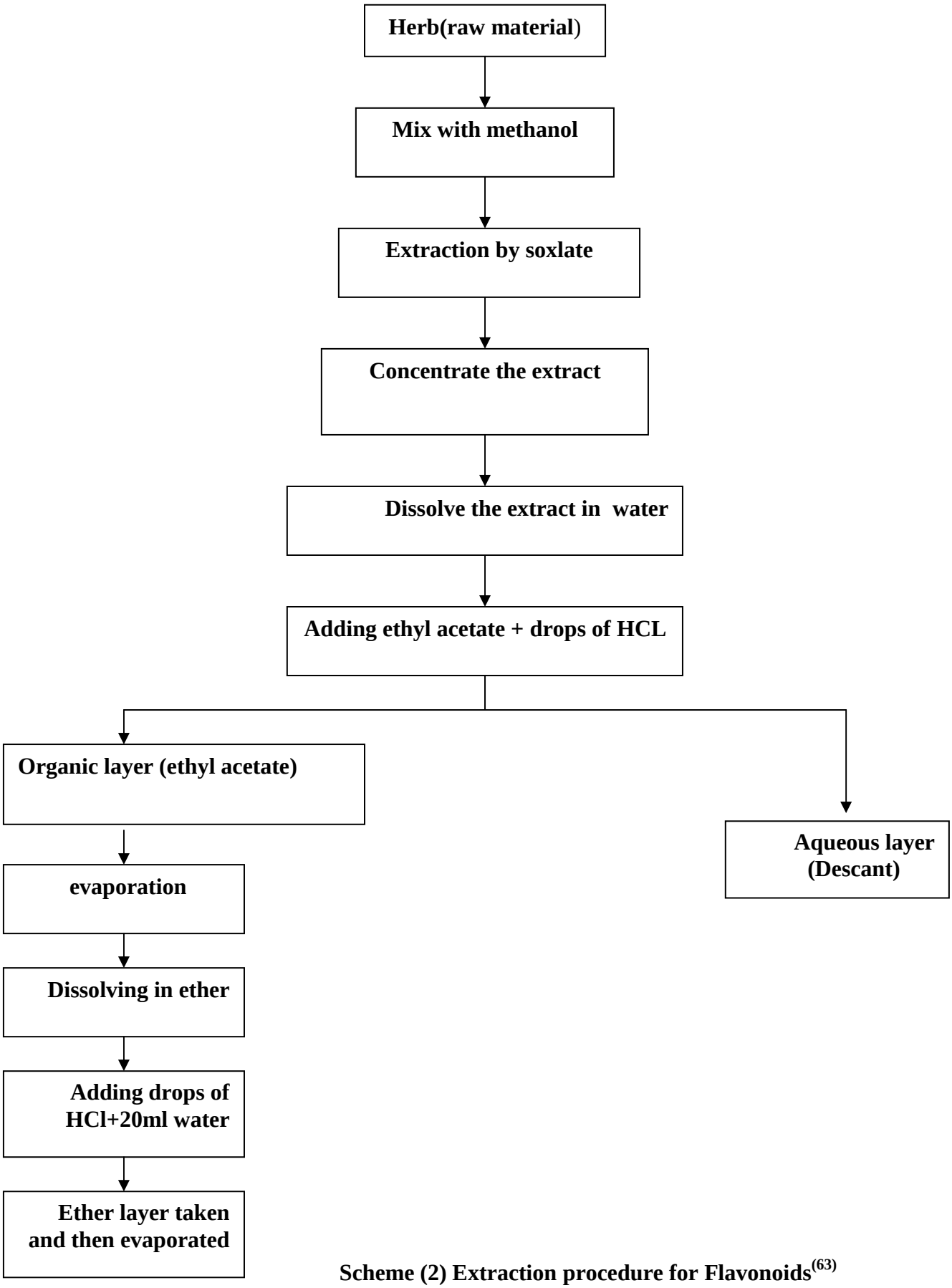
2.4. Atropine extraction;

After extraction of total alkaloids, atropine was obtained by;

1. Dissolving the total alkaloids in 30 ml chloroform and then warming gently with stirring to reduce the enantiomerism of the compound .
2. Evaporate chloroform .
3. Dissolving the total alkaloids in 5% alcoholic H_2SO_4 and then acidification with NaHCO3 to reach pH 5-6 then extract again with 25 ml chloroform to obtain atropine [22]

2.5. Flavonoids extraction:

A 10 gram plant sample was taken and mixed with 50 ml methanol and extracted using soxlate for 6 hours. The extract was concentrated by heating on a water bath to give gummy form.. The extract was then transfered to a separatory funnel and 15ml water was added and shacked with 15 ml ethyl acetate .The Ethyl acetate extract was then acidified with drops of HCl in aqueous media and then shacked with 25 ml ether. The ether layer was taken and evaporated to get crude flavonoids. The scheme (2) show the extraction procedure[63]



Scheme (2) Extraction procedure for Flavonoids[63]

2.6 Instruments and equipments

2.6.1. Melting point measurements:

Melting points were recorded on hot stage Gallen kamp melting point and uncorrected.

2.6.2 Ultraviolet absorption Spectra (UV-VIS):

The ultraviolet spectra were measured using SHIMADZO 1085 spectro-photometer (Japan) and wavelength scanned between 200 -1100 nm using ethanol as a solvent.

2.6.3. High performance liquid chromatography (HPLC) measurements:

Separation was done using HPLC model Shimadzu LC-6A (Japan) with reversed phase DB C-18 (250 × 4.6 mm) column silica as stationary phase. Flow rate was 1ml/min, detection UV at 254 nm at ambient temperature.

2.6.4. F.T.IR:

KBr disk of the materials were measured with Shimadzu Fourier transforms infrared model FTIR 8300 (Kyoto, Japan) .

2.6.5. pH measurement:

pH were measured for the herb extract by taking 2 gm from the crude powdered and extracted in 25ml distilled water.

2.6.6. Atomic absorption:

Element concentrations measurements were done by using Varian Tectron AA-775 atomic absorption spectrophotometer for the determination of Cd, Cu, Mn, Fe, Mg, Ca, K and Zn.

2.6.7. Thin layer chromatography (TLC):

Ready to use plates of silica. Pre-coated 20×20 cm and 0.25 mm thickness (Silica gel 60 F 254 E. Merck Darmstadt. Germany) were used

A- preparation the plates for analysis:

The plates were activated at 110 C^0 for one hour before use. The solvent was allowed to rise 15 cm to give good separation. The solvent system was placed in glass tank (Developing chamber) .

B-Spotting the sample:

10µ L from the samples and standard solutions were used as a spots on the starting point that is 2 cm from the base of plate.

C-Developing of the spots:

Spots were detected on the plates by viewing under short wavelength by using UV light and also detection was done using iodine.

2.7. Chemical Tests

2.7.1 Alkaloid tests

Alkaloid obtained from boiling 10g sample of crude herb with (50ml) distilled water and few drops of 4% HCl after that filter and cooling the solution. A 1ml aliquot of the filtrate was examined by the following testes:

2.7.1. a Dragen-Droff test for Alkaloids

Dragen droff test used to show plant extract free from alkaloid or not using Wagner method.[103] as followed:

Preparation of Dragen droff test:-

The first solution was prepared by dissolving 2g of bismuth nitrate $Bi(NO_3)_3$ in 25ml acetic acid and then 100ml of distilled water were added. Second solution was prepared by dissolving 15g potassium iodide KI in 100ml of distill water. A 10ml from first solution was mixed with 10ml of second solution and the final solution was completed by adding 20ml acetic acid and 100ml of distilled water this final solution represented Dragn-droff test.

A 2ml of extract solution was transferred in test tube and add to it about 3ml of Dragen droff test and note the presence of orange color which confirm that plant extract contained alkaloids.[50]

2.7.1. b Mayer reagent

This reagent was prepared using two solutions

Solution A was prepared by dissolving 0.7 g Hg_2CL_2 in 30 ml distilled water

Solution B was. Prepared by dissolving 2.5g KI in 5 ml distilled water

Both solutions were then mixed, and the volume made up to 50 ml with distilled water.(50)

2.7.1.c Wagner reagent

This reagent was prepared by dissolving 0.5 g KI in 5 ml distilled water and adding 0.375 g I_2 and mixed until dissolving. The volume made up to 25 ml with distilled water.(50)

2.7.2. Flavonoids tests:

Solution (A) was prepared by shaking 15 g of plant powder in 25 ml methanol (95 %), and solution (B) was prepared by adding 15 ml of ethanol (50 %) to 15 ml of potassium hydroxide solution. Mixing equal quantities of both solutions. The presence of yellow color refer to the presence of flavonoids.(56)

2.7.3.Tanins test

A 2 g of Herb powder was mixed with 50 ml distilled water and heating to boiling point then filtered and cooled. Two portions of this solution were taken:

1. To the first solution 7 ml of 1 % $Pb(NO_3)_2$ was added, gelatin precipitate was formed which is as indication for the presence of Tannins.
2. The second solution 10 ml of 1 % $FeCl_2$ was added .Bluish green color was obtained which is an indicatition for presence of Tanins.(77)

2.7.4.Resins test

Adding 10 ml ethanol to 1 g of herb powder and stand for 2 minutes in a water bath. The solution was filtered and 50 ml distilled water acidified with 4 % HCl was added. The appearance of turbid solution is an indication for resin[77]

2.7.4. Saponins test

Aqueous extract of herb powder taken and shaken in test tube until appearance of foam.[77]

2.8. Antibacterial activity measurement:

Antibacterial activity were measured in vitro using paper disc method (agar plate diffusion method) against two pathogenic microorganism viz., *Staph. aureus* (Gram + ve) and *E. coil* (Gram – ve). In this method, a standard 5mm diameter sterilized filter paper impregnated with the water extract plant (5mg / ml, 10mg/ml, 15mg/ml, and 20mg/ml) was placed on agar plate seeded with the test organism. The plates were incubated for 24 hours at 37^{o} C.

الخلاصة

تم خلال هذا البحث دراسة كيمائية على نبتتين عراقيتين هما الداتورة (*Datura stramonium*) و ست الحسن (*Atropa belladonna*). تم استخلاص وعزل الاتروبين الذي هو من القلويدات. عملية الاستخلاص تمت باستخدام الميثانول كمذيب للاستخلاص باستخدام جهاز التصعيدالعاكس. شّخص الاتروبين بواسطة قياس درجة الانصهار التي وجدت (١١٣–١١٦س˚) في ست الحسن وفي نبات الداتورة (١١٤–١١٨س˚) وهي مقاربة إلى المادة القياسية (١١٤–١١٦س˚).كما تم استخدام مطيافية الأشعة تحت الحمراء(IR)، مطيافية الأشعة فوق البنفسجية- المرئية(UV-Vis)، تقنية كروماتوغرافيا السائل العالي الاداء(HPLC)، وكروماتوغرافيا الطبقة الرقيقة (TLC) في عملية تشخيص. الاتروبين.

باستخدام تقنية كروماتوغرافيا السائل العالي الأداء(HPLC) وجدت مركبات فلافونويدية جديدة بتراكيز قليلة جداً.حيث وجد الرتين والكيوريسيتين وجدت في نبات الداتورة بينما الكاتكين وجد في نبات ست الحسن.

كروماتوغرافيا الطبقة الرقيقة(TLC) كذلك أثبتت وجود المركبات بواسطة مقارنة قيم عامل الاحتجاز للمركبات المستخلصة مع القيم القياسية.

تمت دراسة الفعالية البايولوجية للمستخلصات المائية لكلا النباتين بتراكيز مختلفة ضد نوعين من البكتريا وهما البكتريا الموجبة الصبغة ***Staphy.Aureus*** والبكتريا سالبة الصبغة ***Esch.coli***. وقد دلت النتائج على إن المستخلصات المائية اظهرت فعالية بايولوجية ضد النوعين.