Sudan University of Science and Technology

College of Science

Department of Scientific Laboratories – Chemistry

Extraction of Volatile oil and Flavonoids from Henna leaves

استخلاص الزيت الطيار والفلافنويدات من أوراق الحنة

Thesis submitted in Partial Fulfillment of the Requirements for The award of the Degree of honour B.Sc in Chemistry

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الآية :

قَالَ تَعَالَى:

﴿ ٱقۡرَأۡ بِٱسۡمِ رَبِّكَ ٱلَّذِي خَلَقَ ۝١ خَلَقَ ٱلۡإِنسَٰنَ مِنۡ عَلَقٍ ۝٢ ٱقۡرَأۡ وَرَبُّكَ
ٱلۡأَكۡرَمُ ۝٣ ٱلَّذِي عَلَّمَ بِٱلۡقَلَمِ ۝٤ عَلَّمَ ٱلۡإِنسَٰنَ مَا لَمۡ يَعۡلَمۡ ۝٥ ﴾

صدق الله العظيم

سورة العلق-الآيات من ١ - ٥

Dedication

Special dedication to

our family's members that always,

love and stand beside us.

ACKNOWLEDGEMENT

Praise is to god for his help to complete this final year project as one of our requirement to complete our study.

First and foremost, we would like to extend our deepest gratitude to all the parties involved in this research.

her ||First of all, a special thanks to our supervisor Dr. Dalia Mohammed Osman for willingness in overseeing the progress of our research work from its initial phases till the completion of it. We do believe that all best advices and comments are for the benefit of producing the best research work.

Secondly, we would like to extend our word of appreciation to all staff in the lab especially all teaching for their guidance and valuable advices during the experiment of this research. we do believe, commitments and comments are for the benefit.

ABSTRACT

This research is a bout studying some physical properties of a sample of henna leaves, volatile oil and flavonoids were extracted and then analyzed.

Result of detecting some physical properties show: moisture (11%), Ash (10.1265%).

The extraction of volatile oil was done by hydrodistllation and the percentage of the volatile oil was (0.06610 v/w).

Absolute ethanol used to extract flavonoids and then detected by paper chromatography, ferric chloride and vanillin test and the results were positive to the presence of flavonoids. Paper chromatography technique used to isolate three flavonoids from alcoholic extraction and the results showed the presence of three flavonoids.

The three flavonoids were analyzed by using UV and IR the result showed the presence of phenolic groups

The activity of isolated flavonoids and volatile oil against some types of bacteria and fungi were tested and gave weak results because of the low concentrations.

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مستخلص البحث

يختص هذا البحث بدراسة بعض الخواص الفيزيائية لعينة من أوراق الحنة تم استخلاص الزيت الطيار والفلافنويدات وتحليلها .

نتائج تحديد بعض الخواص الفيزيائية أوضحت الاتى :

الرطوبة (11%) والرماد (10.1265%) ، تم استخلاص الزيت الطيار بجهاز Hydrodistallion و تم تحديد نسبة الزيت الطيار (0.066% v/w) .

استخدم الابثانول المطلق لاستخلاص الفلافنويدات وتم الكشف عنها باستخدام كروماتوجرافيا الورقة ،كاشف كلوريد الحديد (III) بالاضافة الى الفانيلين وقد اعطت نتائج ايجابية لوجود الفلافنويد ،استخدمت تقنية كروماتوجرافيا الورقة لفصل الفلافنويدات من المستخلص الكحولي حيث اوضحت النتائج وجود ثلاثة فلافنويدات وتم تحليل الفلافنويدات بواسطة جهاز UV و IR حيث اوضحت النتائج وجود الزمر الفينولية ، وتم اختبار فعالية الفلافنويدات المستخلصة والزيت الطيار كمضاد لبعض انواع البكتريا والفطريات واعطى نتائج ضعيفة نسبة لانخفاض التراكيز المستخدمة .

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CHAPTER ONE

Introduction

1-Introduction

1.1- Henna (Lawsonia inermis):

1.1.1-Definition of henna:

Lawsonia inermis linn is a perennial plant commonly called as henna, henna grows better in tropical savannah and tropical arid zones, in latitude between 15 and 25 degree, produces highest dye content in temperature between 35_45 °C degree [1].

Henna leaves are very popular natural dye to colour hand, finger, nails and hair [1].

For centuries, henna leaves were renowned as the most extensively used natural hair dying and tattooing agent in many civilization and cultures, the

Word henna which means “to become queen “is indicative of something highly elegant, the plant extract or its purified compounds exhibit a variety of biological activities [6].

Lawsonia inermis is much branched glabrous shrub or small tree 2-6 meter in height, which may be spiny. Bark greyish-brown, unarmed when young, older plants with spine-tipped branch lets. Young branches quadrangular, green but turn red with, age. Leaves opposite, entire, sub sessile, elliptic to broadly lanceolate, (1.5-5 x 0.5-2) cm, glabrous, acuminate; veins on the upper, surface, depressed.[1].

Flowers small, white, numerous, in large pyramidal terminal cymes, fragrant, 1 cm across, 4 petals crumpled in the bud. Calyx with 2-mm tube and 3-mm spread lobes; petals orbicular to obovate, white or red;

stamens 8, inserted in pairs on the rim of the calyx tube; ovary 4 celled, style up to 5 mm long, erect. Fruits small, brown, globose capsules 4-8 mm in diameter, many-seeded, opening irregularly, split into 4 sections, with a persistent style. Seeds 3 mm across, angular, with thick seed coat [1].

1.1.2- Classification of Henna:

There are many types of henna like Neutral henna and Red henna [1]:

Table1: Scientific classification of Henna :

Kingdom	Plantae
Order	Myrtales
Family	Lythraceae
Genus	Lawsonia
Species	Inermis

1.1.3-Geoghraphic contribution of Henna:

The origin of Lawsonia *inermis* is unknown. Linguistic evidence supports an origin in the area of Baluchistan (Iran/Pakistan) to western India, where it can still be found growing in the wild. From there it would have

spread eastward to the rest of India and Indonesia, and westward to the Middle East where it became one of the important plants of Islam. It was, however, already mentioned in the Bible for its perfume and in Ancient Egypt [1].

1.1.4- Physical Properties of Henna:

Form: powder

Colour: greenish brown

Odour: light

Solubility in water: insoluble

Stability and reactivity: thermal

Decomposition/condition to be avoided: avoided Sunlight

Toxicological information: further toxicological effects: product is not

Toxic [1].

1.1.5- Chemical Properties of Henna:

The dyeing agent in henna is Lawson or 2-hydroxy-1,4-naphthoquinone (naphthalene Dione), which is present in dry leaves at a concentration of 0.5–2%. It attaches itself strongly to proteins, and as a result the dye is very fast. Other components in henna such as flavonoids (luteoline, acacetine) and Gallic acid contribute as organic mordant to the coloring process; carbohydrates (vegetable gelatin, mucilage) give the henna paste a suitable consistency to attach to hair and possibly they also play

a role in the penetration of Lawson into the hair and other tissues. The stem contains variable amounts of tannins. On steam distillation, the flowers yield 0.01–0.02% essential oil (henna oil), mainly consisting of α- and β-ionone, which can be used as a basis for perfumes. The seeds contain about 10% of a non-drying, viscous oil, composed mainly of oleic, linoleic and stearic acids[4].
Henna showed anti-inflammatory, analgesic and antipyretic effects, but it may cause side-effects such as hemolytic anemia in cases of glucose-6-phosphate dehydrogenase enzyme deficiency. Henna extracts also showed molluscicidal and trypanocidal activities. A leaf extract showed antitumor and tuberculostatic effects in tests with mice. It exhibited a broad fungi toxic spectrum when tested against various ringworm fungi, which was attributed to Lawson. In India henna preparations showed antifertility activity [4].

1.1.6- Uses of Henna:

Henna is one of the oldest cosmetics in the world and its leaves are used to color the fingernails, to paint or decorate the palms of the hands and the soles of the feet, and to dye the hair, written records of the use of henna date back more than 2500 years,henna is of great importance in Islam, where it is used in many ceremonies, especially marriage, in the belief that henna purifies and protects; designs differ per region and culture, indicating for example good health, wisdom and spiritual enlightenment. Henna is used universally as a basis for hair dyes [4].

The leaves are used as aprophylactic against skin diseases, they are used externally in the form of baste or decoction against boils, burns, bruises and skin inflammations a decoction is used as gurgle against sore throat, the roots of this plant are useful in burning sensation, leprosy [2].

The major phytochemical constituent of henna, Lawson, was found to possess significant anti-inflammatory, analgesic and antipyretic activities[2].

1.2-Natural Products:

A chemical compound or substance produced by a living organism that is found in nature.in the broadest sense, natural products include any substance produced by living organism, natural products can also be prepared by chemical synthesis [12].

Main class of natural products:

- Carbohydrates
- Lipids
- Proteins
- Nucleic Acids

Carbohydrates: A broad category of chemical compounds, also referred as sugars the most a class of abundant class of bio- organic molecules of earth Although relatively low in human, it constitutes about 75% by mass of dry plant materials [5].

Lipids: abroad category of chemical compound, also referred as fat. most of those products are those products are non-polar fat, oil, or wax. that does not (or poorly) dissolves in water [5].

Proteins: a broad category of chemical compounds of amino acids, includes Amino acids, polypeptide, enzymes [5].

Nucleic acids: a category of complexes chemical compounds involved in the transmission of genetic information (DNA) and its transfer as information to the cell(RNA) [5].

1.3-Volatile oils:

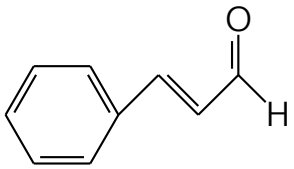
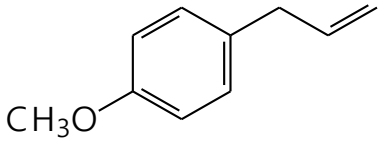
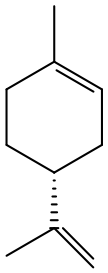
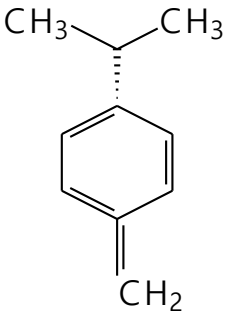
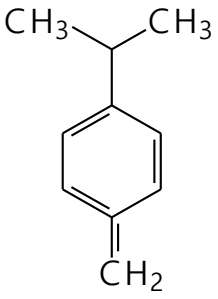
Volatile oils are generally mixture of hydrocarbon and oxygenated compound derived from these hydrocarbon, in some oils the hydrocarbon are predominated and only limited amounts of oxygenate constituents are present in other the bulk of oxygenate compound, the odour and taste of volatile oils are mainly entwined by these oxygenated constituents which are to some in origin, a smaller number of volatile oils contain principally aromatic derivatives mixed with [7].

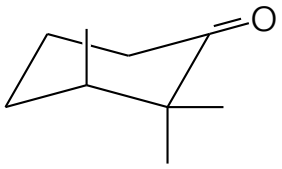
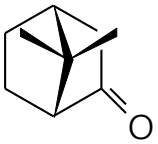
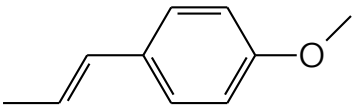
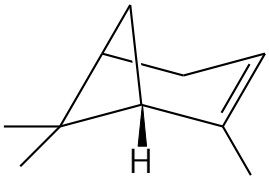
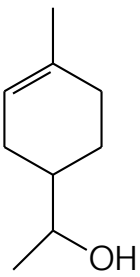
The simpler mono-and sesqui-terpnoid are the chief constituents

of volatile oil, the volatile oil is obtained from tissue of certain plants. Terpnoids are all based on the isoprene molecule and their carbon skeletons are build up from the union of two or more of the unit.,they are classified according to whether contain (c_{10}) (c_{15}) such units,they range from essential oil components, the volatile mono and sesquiterpens through the less volatile diterpenes to the in volatile triterpenes and sterols and carotenoid pigment, although are derived biogenetically from the molecule of isoprene, which does occur as natural product [7].

The formula of some common constituents of pharmaceutical volatile oils are given in figure [7].

Table 2:some common constituents of pharmaceutical volatile oil

Name of volatile oil	Structure of volatile oil
Cinnamaldehyde	
Estragole	
Limonene	
Beta – phellandrene	
Alpha- Phellandrene	

Fenchone	
Myrecene	
Camphor	
Anethole	
Alpha-pinene	
Alpha – terpineol	

1.4- Flavonoids:

1.4.1- Definition:

Flavonoids contain conjugated aromatic systems and thus show intense absorption band in UV and visible regions of spectrum or this reason

flavanone are intensely colour, providing a spectrum of colours occur as dimers, in which two classes of flavonoids, mostly flavones and flavanones are joined together. coupling may be bound together erent types of flavonoids, they may be bound together directly through their carbons and most often by C8 and C6 by COC inter flavone link [11].

Flavonoids constitute one of the most characteristic classes of compound in higher plants, many flavonoids are easily recognized as flower pigment in most families [flowering plant]. however, their occurrence is not restricted to flower but include all parts of the plant, root, heartwood, sapwood, bark, stem, leaf, fruit and seed, some kinds of flavonoids are more characteristic of certain tissues[table2] which are present in all vascular planet, some classes are more widely distributed than other, while flavones and flavones are almost universal [11].

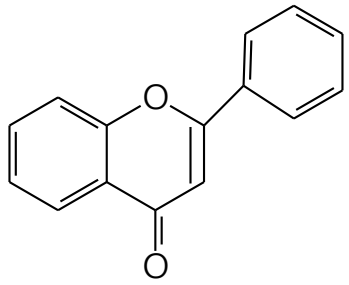
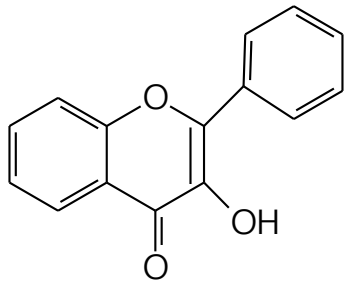
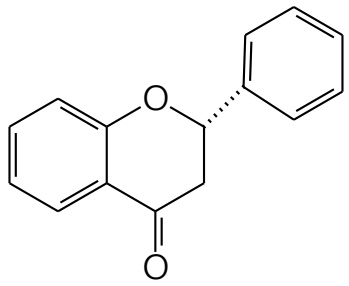
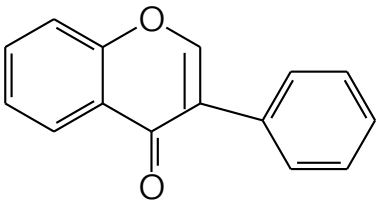
The presence of flavonoid in plant is largely influenced by genetic factors such as germination, degree of ripeness, processing and storage also influence the content of plant phenolic, flavonoids are responsible for the coloration of the flowers fruits and some time the leaves, colourless flavonoids are also abundant and many function as cop-pigments, the flavonoids play an important role in the protection of the plant against the harmful and damaging effect of UV radiation [11].

Flavonoids have significant impact on various species of plant biology, the exhibit a wide range of functions physiology bio chemistry, and ecology, moreover, for long time flavonoids constituted useful tools in physiology, biochemistry, and ecology, moreover, for long time flavonoids constituted useful tools in phylogenetic studies. they are

believed to protect humans by providing protection against certain form of cancer and reduction of cardio vascular disease [11].

Flavonoids possess strong anti -oxidative activity as well as other potential beneficial effect including anti-inflammatory, anti-viral, anti-atherosclerotic., anti-cancer, and anti-osteoptic effects [11].

Table 3: characteristic properties of the different flavonoid classes:

Sub-class of flavonoids	Basic structure of flavonoids	Characteristic properties
Flavone		Represent the root of the flavonoid , differ from flavonol in lacking a 3-OH substituent
Flavonol		Represent the most common compound differ from flavons in having a 3-OH substitution
Flavanone		Differ from all classes of flavonoids in lacking the double bond in 2,3 position
Isoflavone		Isomeric with flavones having the B-ring attached at the 3-position in flavones instates of 2-position in flavones

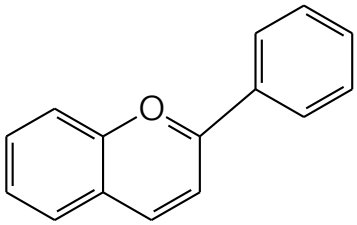
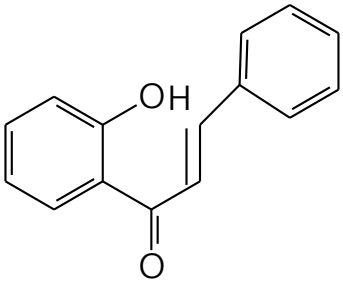
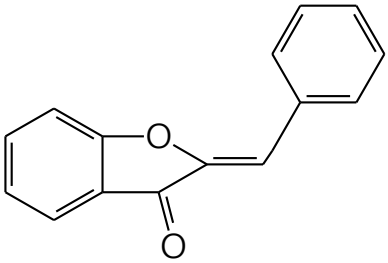
Anthocyanin		Differ from all class of flavonoids in lacking the carbonyl group at 4-position
Chalcone		Isomeric with flavanone having the open chain in flavanone (ring c)
Aurone		Differ from all class of flavonoids having 5-membred ring-c instated six-membred ring in certain class

Table 4: colour properties and occurrence of the different flavonoid classes.

Flavonoid	Occurrence	Colour and nature
Flavone	Found in all part of plant, widespread in flower and leaves.	Yellow colour
Flavonol	The same as flavones	Yellow colour
Flavanone	The same as flavones	Colourless
Isoflavone	Found in root;only common in one family; the leguminacea	Colourless substance
Anthocyanain	Flower pigments; also in leaves;, fruits petals and other tissues	Scarlet, mauve ,blue ,pink and violet
Chalcone	Flower pigments, occasionally present in other tissues	Bright yellow colour
Aurone	Flower pigments ;widespread in leaves , fruits and bark wood	Bright yellow colour

1.4.2-Extraction of Flavonoids:

After drying and grinding plant there are several ways to extract flavonoids referred to some references, it is demodulation solution of alcohol), methanol, or a combination of both (and this the more ways to extract flavonoids, followed by the extraction of an optional means of media after getting rid of the alcohol concentrate. It is more Solvents commonly used for this purpose ethyl acetate (Acouet) and normal butanol (n- BuoH), may be used others solventssuch as normal hexane, chloroform, ether oil, dual methane [11].

1.4.3- Detecting of Flavonoids:

Testing is done, by the way (AL- kazraji1991) as added 1 ml of reagent of potassium hydroxide Alcoholic (Ethanolic [5N] KOH) to 1 ml of the extract, and when you see the yellow precipitate is the result Positive, this is evidence of the presence of flavonoids [11].

1.4.4- Classification of Flavonoid Compound:

Flavonoids are sub-category of plant phenolic, they are widely distributing and characterized by the same basic structural element flavonoids are present in highly classes of secondary plant metabolites with about9000structure [11].

Classification of flavonoid type in plant tissue is based initially on study of solubility properties and colour reaction this is followed by a one-dimensional chromatographic examination of hydrolysed plant extract

and a-tow-dimensional chromatographic separation of direct alcoholic extract[11].

Flavonoids can be divided into at last 7different classes depending on their basic chemical structure. flavonoids within certain group, can be further subdivided into classes flavonoids are classivied by several methods [11].

In the first methods flavonoid are divided into two major group according to the degree of saturation of the central hetero cyclic ring, the un saturated group have planer geometry, and the saturated groups are characterized by the absence of the 2,3-double bonds and include flavanones and flavones[11].

These molecules normally have one or more chiral centre in the second method flavone odes are classified according to the substitution pattern of ring [11].

1.4.5- Importance of Flavonoids:

In leaves Flavonoid are increasingly believed to promote physiological survival of the plant, protecting it from for example fungal pathogens and UVradiation [13].

Flavonoids are important antioxidants, and promote several health effects. Some of the activities attributed to flavonoids include: anticancer, anti-inflammatory, anti-viral and anti-allergic, one flavonoid called quercetin ca-n help to alleviate eczema, sinusitis, asthma, and hay fever [8].

Flavonoids support the cardiovascular and nervous system, because they also help support detoxification of potentially tissue damaging molecules their intake has often, although not always, been associated with decreased certain types of cancer, however it is important to note that amount of flavonoids required to provide the above health benefits is not certain [10].

Some studies have shown that flavonoid intake is inversely related to heart disease, with these molecules inhibiting the oxidation of low-density lipoproteins and therefore reducing the risk of Atherosclerosis developing.

Flavonoids are used by botanists for taxonomical classification, they regulate plant growth by inhibition of the exocytosis of auxin induction of gene expression, and they influence other biological cells in numerous way[9].

1.4.6- Paper Chromatography(PC):

The technique of paper chromatographic is a common one in the field of flavonoid analysis and separation [11].

PC is suitable for the separation complex mixture of all type of flavonoid It is convenient for isolating of both small and relatively large amounts and is associated with the low cost of the necessary equipment and material.

one of the main advantages of pc is the great convenience of carrying out separation simply on sheets of filter paper which serve both as the medium for separation and as the support. another advantage is the considerable re reducibility of R_F determined on paper, so that such measurements are valuable parameters for used in describing new plant compound indeed, for substance such as anthocyanin, which do not have other clearly define physical properties, the R_F is the most important means of describing and distinguishing the different pigments [11].

Most flavonoids as coloured spot on paper chromatogram when viewed UV-light, and fuming with ammonia often produces significant changes in these colours. often reaction with chromogenic reagent which is used as a spray is extremely useful [11].

1.4.7- Thin _layer chromatography (TLC):

TLC is a technique which has developed rapidly. however, it is complementary to PC in that it provided new media for separation of flavonoids on small scale and permits the use of a wider variety of detecting reagent , the special advantage of TLC compared to PC include speed, versatility and sensitivity. the greater speed of TLC is due to the more compact nature of adsorbent when spread on plates and is advantage when working with labile [11].

1.5- Analysis:

1.5.1- UV/VIS Spectroscopy:

The absorption or reflectance in the visible range directly affects the perceived colour of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transition from the excited stated to the ground state, while absorption measures transitions from the ground state to the excited state [14].

1.5.2- Infrared Spectroscopy:

Infrared spectroscopy is certainly one of the most important analytical techniques available to today's scientists. One of the great advantages of infrared spectroscopy is that virtually any sample in virtually any state may be studied. Liquids, solutions, pastes, powders, films, fibers, gases and surfaces can all be examined with a judicious choice of sampling technique. As a consequence of the improved instrumentation, a variety of new sensitive techniques have now been developed in order to examine formerly intractable samples. Infrared spectroscopy technique is based on the vibrations of the atoms of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule [3].

1.6-Aims of this study:

-Extraction of volatile oil from henna leaves.

- Extraction of Flavonoid.

-Analysis of Flavonoid by UV/VIS and IR spectroscopy.

-Determine the activity of Flavonoids against bacteria and fungi.

-Evaluation of extracted volatile oil and flavonoids for their antimicrobial potential.

CHAPTER TWO

Materials, Instruments and Method

2-Materials, Instruments and Method

2.1- Materials:

2.1.1- sample:

Fresh leaves Henna (Lawsonia interims) were collected from Omdurman (ombada32).

2.1.2- Test organism:

2.1.2.1- Bacterial microorganisms:

Bacillus subtilis	NCTC 8236 (Gram + ve bacteria)
Staphylococcus aureus	ATCC 25923(Gram +ve Bacteria)
Escherichia coli	ATCC 25922(Gram -ve bacteria**)**
Pseudomonas aeruginosa	ATCC 27853 (Gram -ve bacteria)

2.1.2.2- Fungal microorganisms:

Aspergillus niger	ATCC9763
Candida albicans	ATCC7596

2.1.3- Chemicals and solvents:

- Absolute Ethanol (99,9%)
- n- Hexane(0.673g/cm^3)
- Acetic Acid(1.05g/cm^3)
- 1-Propanol (0.803g/cm^3)
- Vanillin for Synthesis

- Ferric chloride

-Concentrated Nitric Acid (69%)

2.2-Instruments:

- Hydro distillation system (Shaven seal for volatile oil made in chine lob chimes)

- Oven (made in china by binder)

- Furnace (UK by Scott Science)

- UV spectroscopy (JENWAY,6505 UV/VIS, British).

- IR spectroscopy (Thermo Nicolet 300).

2.3-Method:

2.3.1-Sampling:

The leaves were washed with water to remove all dust particles. after complete drying at room temperature then leaves were powdered.

2.3.2-Methode of determination some physical properties of Henna leaves:

2.3.2.1-Method of determination of moisture :

2.085 gram of the sample dried in oven for 24 hours at 100 C° and cooled then weighed.

2.3.2.2-Method of determination of ash percentage:

The residue from moisture was ignited at 500C° until the ash was grey then put in desiccator and weighted.

2.3.3- Method of Extractions:

2.3.3.1- Method of Extraction of volatile oil:

fresh leaves of henna extracted by hydro distillation system.

Fresh henna leaves (150g) were extracted for the volatile oil by hydrodistillation method using Clevenger apparatus.

The leaves are fully submerged in a round-bottomed flask, equipped with a condenser and boiled, using a heating mantle, to produce vapours which condensed yielding a two phase of oil and water returned to the round- bottomed flask and the oil was separated. Extraction was carried out at the boiling point temperature of the water for about 4 hours.

2.3.3.2- Method of Extraction of flavonoids:

60 gram of sample was weighted, then about 200ml of ethanol was added and left for three days then the solution was decantation then filtered and left until all ethanol was evaporated

System solvent consist of (4:4:2) (1-propanol: acetic acid: water)

The sample was spotting, and left until separated of three flavonoids A, B, C

2.3.4- Analysis of flavonoids:

2.3.4.1- Chemical test:

2.3.4.1.1-Ferric chloride test:

1ml of ferric chloride was added to 1ml of samples A, B, C.

2.3.4.1.2-Vanillin test:

0.5g of vanillin dissolved by ethanol, 1 ml of Nitric acid was added to samples A, B, C.

2.3.4.2- UV method:

Small amount of samples A, B, C with ethanol put in cuvette and read by UV spectroscopy.

2.3.4.3-IR method:

Small amount of samples A.B.C put on cell read by IR spectroscopy.

2.4.5-Anti-microbial test method:

2.4.5.1-Preliminary phytochemical screening:

The preliminary phytochemical screening of Lawsonia inermis was carried out for the decotion of various Phyto-constituents using standard procedure [13] The following solvents were used for the study, petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. The methanolic extract was found to contain more flavonoids. The preliminary phytochemical screening of methanolic extract reveals the presence of alkaloids, flavonoids, tannins and quinones.

2.4.5.2-Preparation of the test organisms:

2.4.5.2.1-Preparation of bacterial suspensions:

One ml aliquots of a 24 hours' broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37^{o} C for 24 hours. The bacterial growth was harvested and washed off with 100 ml

sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (**Miles and Misra, 1938**). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.4.5.2.2- Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

2.4.5.3-Testing of antibacterial susceptibility:

Disc diffusion method: The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using

Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts.The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Table 5: Anti-Bacterial test for volatile oil and flavonoids:

Zone diameter(mm)	**Micro organisms**			
12-13	Bacillus subtilis	NCTC	8236	Gram +ve bacteria
8-9	Escherichia coli	ATCC	25922	Gram +ve
10	Staphylococcus aureus	ATCC	25923	Gram +ve
12-10	Pseudomonas aeruginosa	ATCC	27853	Gram -ve
8	Candida albicans	ATCC	7596	Fungi
10	Aspergillus higer	ATCC	9763	Fungi

CHAPTER THREE

RESULT AND DISCUSSION

3.RESULT AND DISCUSSION

3.1- Result:

3.1.1- Result of determination some physical properties:

3.1.1.1- Result of determination of moisture:

Moisture percentage in henna equal 11%.

3.1.1.2- Result of determination of ash percentage:

Ash percentage in henna equal 10.1265%.

3.1.2- Result of Extraction:

3.1.2.1- Result of determination of volatile oil in Henna:

Percentage of volatile oil equal 0.066%.

3.1.2.2- Result of separation of flavonoids by Paper Chromatography:

The extracted solution separated to three components A, B, C:

Sample	Observation
A	Pale yellow
B	Light green
C	Deep brown

Table 6: Result of separation of flavonoids by Paper Chromatography

3.1.3-Results of analysis of flavonoids:

3.1.3.1- Result of chemical test:

3.1.3.1.1- Result of ferric chloride with A, B, C:

SAMPLE	Observation
A	Violet
B	Deep green
C	Deep brown

Table 7: Result of ferric chloride with A, B, C

3.1.3.1.2- Result of vanillin test with A, B, C:

Sample	Observation
A	Clear orange
B	Clear orange
C	Clear orange

Table 8: Result of vanillin test with A, B, C

3.1.3.2- Result of R_f :

Sample	R_f values
A	0.88
B	0.80
C	0.691

Table 9: Result of R_f

3.1.3.3-Result of UV spectroscopy:

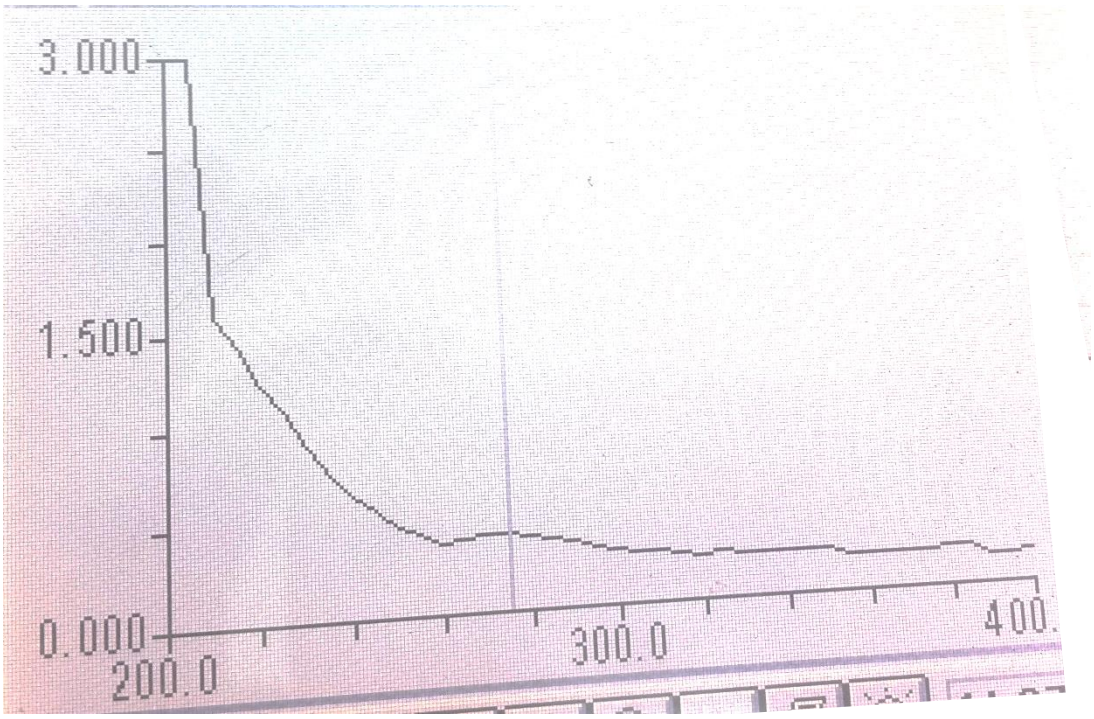


Fig (1) show UV for sample A

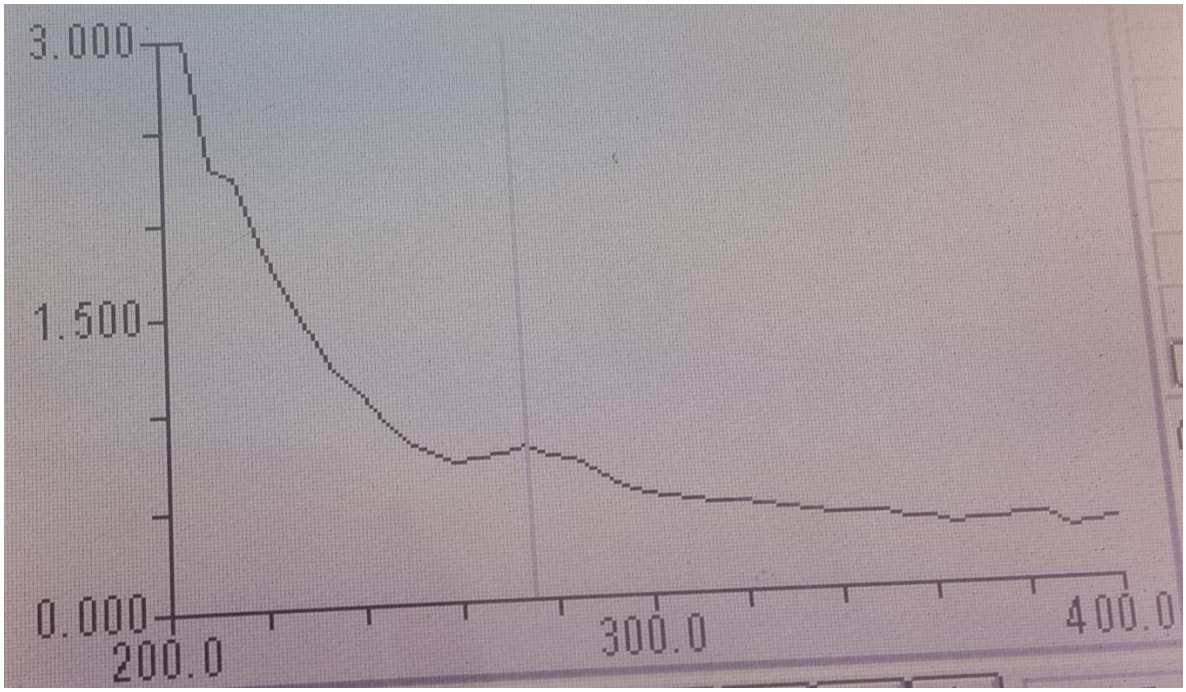
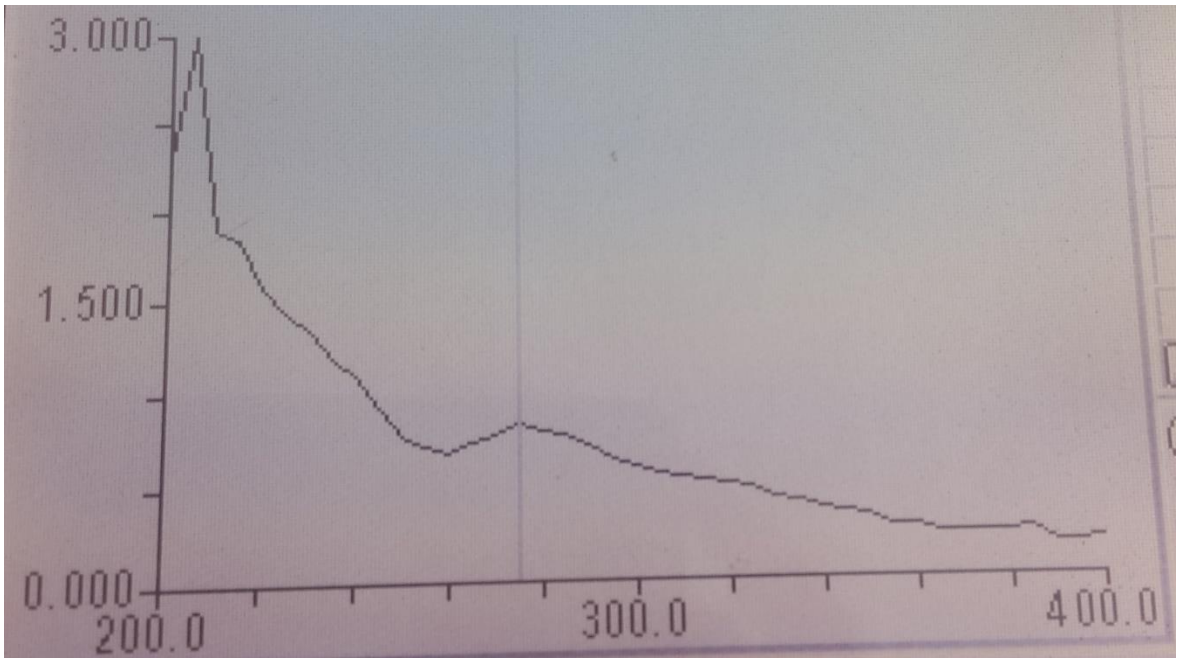


Fig:(2) show UV for sample B



Fig(3) show UV for sample C

3.1.3.4 Results of IR Spectroscopy:

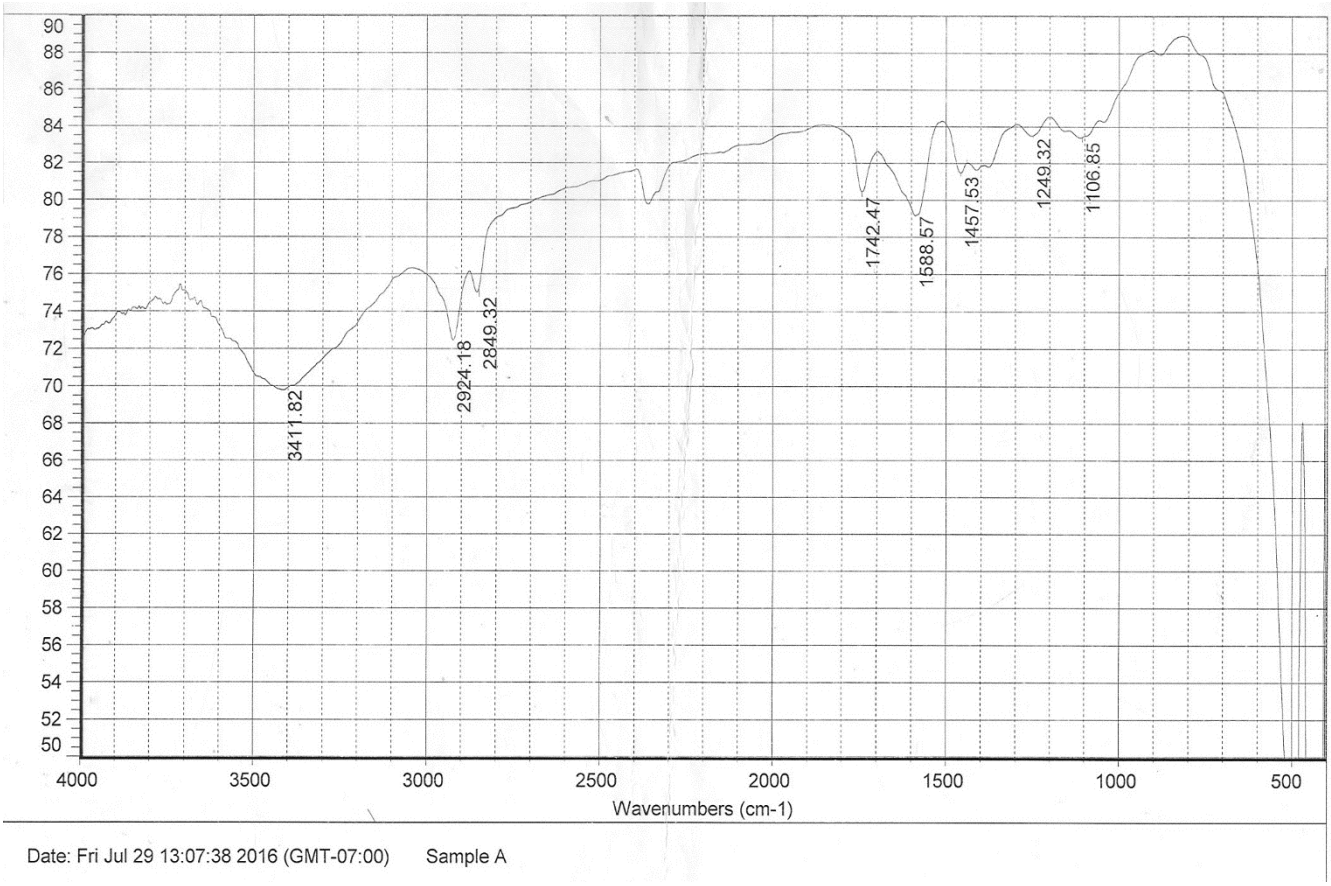


Fig:(4) show compound A.

The spectrum shows the following peaks:

3411.82 (OH)

2924.18, 2849.32 (C-H SP^3)

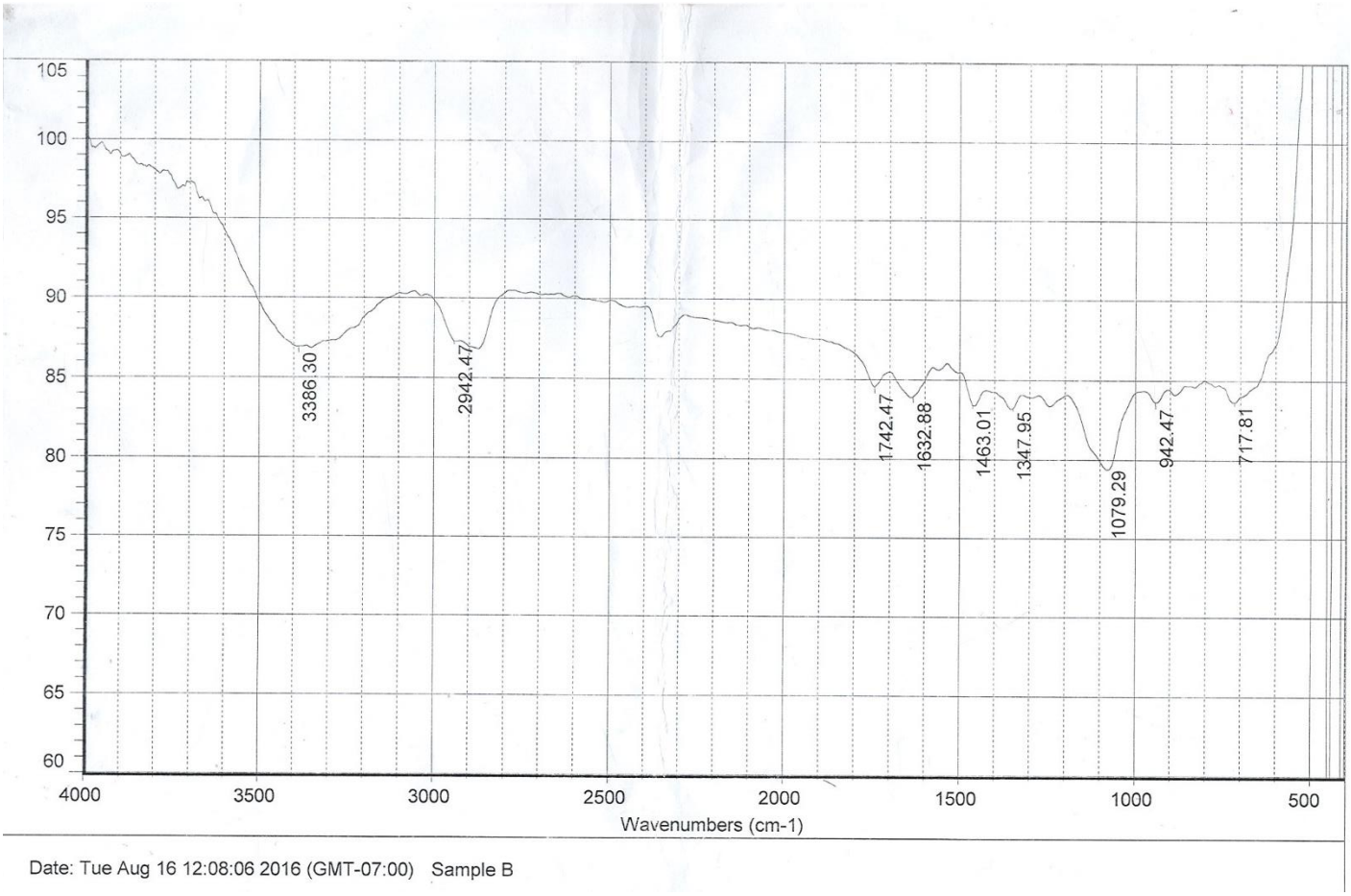


Fig:(5) show compound B

3386.30 (OH)

2942.47 (C-H aliphatic)

1079.29 (C-O)

1463.01 /1632 (Benzene ring)

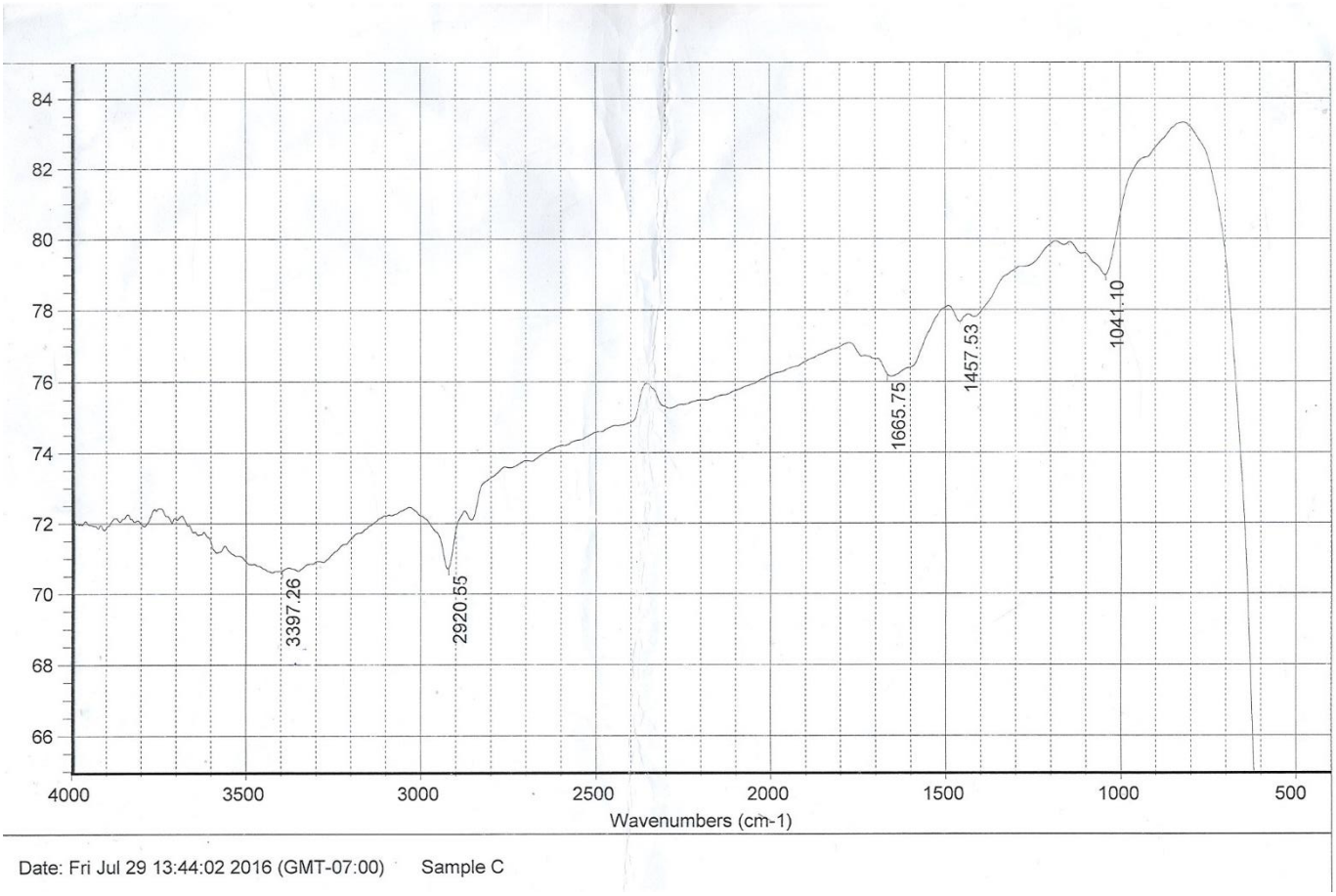


Fig:(6) show compound C

3397.269 (OH)

2920.55(C-H aliphatic)

1041.10(C-O)

1457.53\1665.75 (Benzene ring)

3.2-Discussion:

Percentage of moisture in dried henna leaves (11%) is considered high and according to ash percentage, percentage of organic compounds considered higher than inorganic compounds

Volatile oil percentage in henna leaves was very low. In general percentage of Volatile oil in plants is very low.

The presence of flavonoids in alcoholic extraction was detected by paper chromatography, ferric chloride, vanillin and they gave positive results.

The suitable solvent system to isolate the three flavonoids was the medium polarity system (1-propnol, acetic acid, water), and according to the R_f values the first isolated flavonoid R_f is 0.88 because of its less polarity and the high polarity is belonging to

the third flavonoids R_f is 0.691 The other solvent system didn't isolate the three flavonoids clearly because of the higher polarity.

Flavonoids were extracted by absolute ethanol and isolated to three flavonoids by paper chromatography and comparing to previous study (Bina.S, Nezam.U, Muhammed.1.C, Bioactive flavonoids from the leaves of lawsonia alba Henna) they discovered three new flavonoids lawsochrysine, lawsochry sinine, Lawson ariginin.

When detecting the presence of flavonoids in the three isolated flavonoids when using ferric chloride coloured solutions were obtained.

Ferric chloride gives violet, deep green, deep brown.

UV/VIS spectroscopy can differentiate between flavonoids with double bonds in position 2-3. The UV spectra of most flavonoids consist of two

major absorption maxima one of which occur in range 220-285nm (Band II), and the other in the range 300-400 nm (Band I).

IR spectrum show the presence of phenolic group, A and B show the presence of another substituent beside flavonoid, the two peak in sample A at 2924.18/2849.32 appear due to C-H aliphatic in different position.

Volatile oil and flavonoids activity show weak activity against bacterial and fungi that because of the low concentration, previous study give promising antibacterial activity against shigella and vibrio cholera and no antifungal activity was detected.

Conclusion:

Volatile oil was extracted from a sample of Henna leaves and the percentage was (0.066%). Flavonoids was isolated from alcoholic extraction to three flavonoids, the three flavonoids were analysed by using UV/VIS and IR spectroscopy. the activity of Flavonoids was tested against bacteria and fungi.

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APPINDEX

Fig: (7) show Alcoholic Extraction

Fig:(8) show after vanillin test

Fig:(9) show Isolated flavonoids

Fig:(10) show result of ant-microbial test