Sudan University of Science and Technology

College of Science

Department of Scientific Laboratories (Chemistry)

Phytochemical screening of Solenostemma argel & extraction & separation of its flavonoid

المسح الكيميائي لنبات الحرجل وإستخلاص وفصل الفلافنويد منه

A thesis submitted for the partial requirements of

B.Sc degree in chemistry

By:

Rayan Adel Abdelgader Saty

Sana Yousof Omer Alhuseen

Hind Mohammed Ahmed Alamin

Supervisor:

Dr. Mohammed Sulieman Ali

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بسم الله الرحمن الرحيم

الإستهلال

قَالَ تَعَالَىٰ:

﴿ قُل لَّوۡ كَانَ ٱلۡبَحۡرُ مِدَادٗا لِّكَلِمَٰتِ رَبِّي لَنَفِدَ ٱلۡبَحۡرُ قَبۡلَ أَن تَنفَدَ كَلِمَٰتُ رَبِّي وَلَوۡ جِئۡنَا بِمِثۡلِهِۦ مَدَدٗا ﴾

صدق الله العظيم

سورة الكهف الأية (109)

Dedication to:

Our parents, sisters and brothers. Thanks so much for all your support encouragement.

Love and respect.

Acknowledgements

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In addition, thanks to Industrial Research Consultation Center. Thanks to our teacher Hassan Alzain for guiding and providingus some materials. Special thanks to all of our friends for their support. Thanks to our family No words can express how grateful I am for all the support

Abstract:

At the present work phytochemical screening , extraction and separation of flavonoid from Solenostemma argel was studied. The result obtained showed that Solenostemma argel contains different medicinal species such as flavonoids, alkaloids and diterpenoids. It also showed that the flavonoid had antibacterial activity. IR analysis indicated that all the characteristic peaks of flavonoid were appeared.

المستخلص

في العمل الحالي تم دراسة المسح الكيميائي وإستخلاص وفصل الفلافنويد من الحرجل.

النتائج المتحصل عليها وضحت أن نبات الحرجل يحتوي على أنواع طبية مختلفة مثل: الفلافنويدات، القلويدات، التربينات الثنائية. كذلك أظهرت النتائج أن الفلافنويد يمتلك فعالية ضد البكتريا.

التحليل الطيفي تم بإستخدام جهاز الأشعة تحت الحمراء. أظهر طيف الأشعة تحت الحمراء جميع الزمر الوظيفية الموجودة في الفلافنويد.

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Introduction and literature review

Chapter one

1.1 Introduction:

Man's dependence on plants for the essentials of his existence has been of paramount importance in his life since the human race began. Although various natural products contribute to his welfare it is the plant kingdom that is most essential to man's well-being, the increased use of plant materials both as a mean to better health and for the treatment of specific aliments is known as herbalism . The plants commonly used by herbalists are referred to as medicinal plants or herbs. Recently, a growing interest has developed by researchers in many countries and many publications have appeared on the subject. Green medicines in all available literature medicinal plants are defined as: the plants which serve as sources of various types of chemical compound with complex structures that has the potential to cure many diseases, while the botanical definition of herb is: the plant whose stem is not woody and persistent. The meaning of the word herb is open to argument by botanists and herbalists [1].

1.2 The reasons of studying natural product:

-Natural products are the source of the most complex and fascinating chemical structures.

-Natural products represent biological diversity.

-Natural products are expressions of the genome.

-Natural products represent biological activity. Whether as single compounds are parts of the natural wealth of the country and can be an important source of livelihood, from agriculture and food, pharmaceuticals and fine chemicals industry.

-natural product can be an effective bridge from tradition to modern scientific developments, including genetics, molecular biology, biotechnology and pharmaceutical [2].

1.3Medical uses of natural products:

Herbal medicines have provided the world's population with safe, effective and low cost medicines for centuries. They have a rich and extensive historical basis in use and study which can be referenced in ancient medical writing. More importantly modern research has validated many of these traditional uses. When integrated into medical care with other medications, herbal medicines can provide consumers and patients with the best chance for maintaining a high quality of life and in some cases, increase their chance for survival. They can also fill therapeutic niches that are not adequately addressed through conventional therapies [3].

The main problem facing the use of herbal medicines is the proof requirement that the active ingredients contained in medicinal plant are useful, safe and effective. This is highly important requirement to get the approval of health authorities and to assure the medical staff and the public with regard to the use of medicinal plants as drug alternatives. The proofs of pharmacological activity that are available at present are mostly based on empirical experience. The scientific and clinical proofs then become the most important priority in order to eliminate the concern of using medicinal plants as drugs for alternative treatment. Therefor it is of vital importance to conduct research or provide scientific proof of pharmacology international collaboration is important for utilization of these herbal medicines as

it would enhance the development of drugs obtained from medicinal plants for the benefit of all [4].

1.4 Natural products classification based on their chemical structure:

It is based on the type of chemical skeleton, so there are:

-Aliphatic or non-aliphatic fatty compounds of open chain as: fatty acids, sugars and great amount of amino acid.

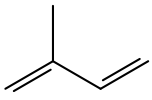
-Acyclic and cycloaliphatic compound as:terpenoids, steroids and some alkaloids.

-Aromatic or benzoic compounds as: phenols, quinones, etc.

-Heterocyclic compounds such as:alkaloids,flavonoids and nucleic acid bases[5].

1.4.1 Terpenoids:

A wide spectrum of "plant products" are duly covered by the terminology 'terpenoid', which is implied to relate all such chemical entities having a common spectacular biosynthetic origin. In a broader prospective, practically all terpenoids are more or less based upon the specific 'Isoprene Molecule' [6]:



-Classification of Terpenoids:

-Mono terpenoids: two isoprene units [$C_{10}H_{16}$].

-Sesquiterpenoids: three isoprene units [$C_{15}H_{24}$].

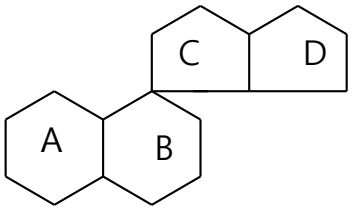
-Di terpenoid: four isoprene units [$C_{20}H_{32}$].

-Tetra terpenoids: eight isoprene units [$C_{40}H_{64}$].

1.4.2 Steroids:

Steroids are among the most important natural products. They are widely kdistributed in animals and plants and have been extensively studied since the isolation of cholesterol. Early in the nineteenth century some of the prominent biologically active compounds are steroids among them are the steroids alcohols (sterols), bile acids, sex hormones, hormones of the adrenal cortex and are cardiac aglycones.

General structural of steroids:



The steroids found in two sources [7]:

-Animals (cholesterol).

-Plants (stigma sterol).

1.4.3 Alkaloids:

Meisner(1819) first and foremost introduced the common and the well-known terminology (Alkaloid) to designate critically and specifically all such natural substances almost interacting like: Base or Alkalis.

Classification of Alkaloids:

An attempt has been made to classify the ‘Alkaloids’ solely based upon the N-heterocyclic rings namely:

-Pyrrolidine alkaloids.

-Pyridine alkaloids (or piperdine alkaloids).

-Pyridine_Pyrrolidine alkaloids.

-Tropane alkaloids.

-Quinoline alkaloids.

-Iso quinolone alkaloids.

-Indole alkaloids.

-Imidazole alkaloids.

These aforesaid eight typical N-heterocyclic basic ring categories of ‘alkaloids ‘ shall now be treated individually in the section that follows and duly exemplified with appropriate examples(s) from each last of alkaloid [8] .

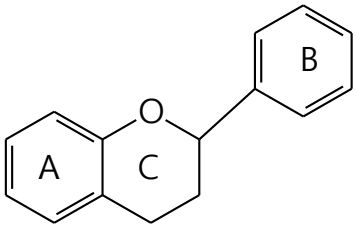
1.4.4 Flavonoids:

The term flavonoid from the Latin word “Flavous” meaning yellow. Is generally used to describe a broad collection of natural products processing 15 carbon atoms , having C_6 -C_3-C_6 carbon frame work , comprising two benzene rings (A and B) linked through heterocyclic pyrane ring [C].

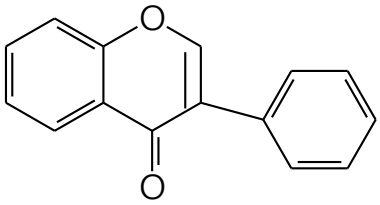
Classification:

There is more classification of flavonoid such as [9]:

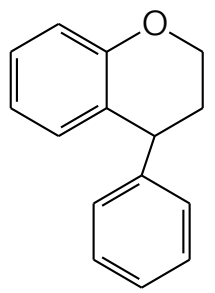
-Flavonoids.



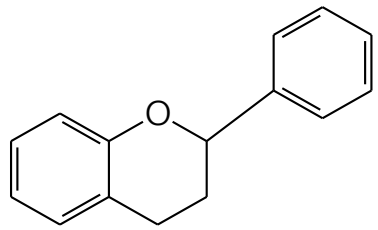
-Isofalvone



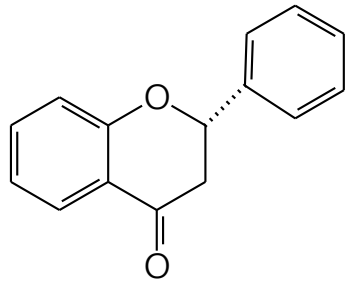
-Neoflavoniod



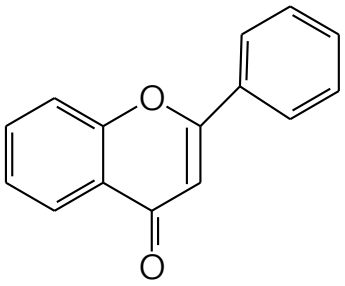
-Flavan



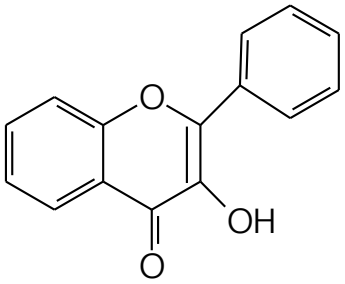
-Flavanone



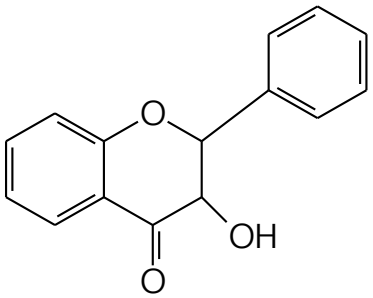
-Flavone



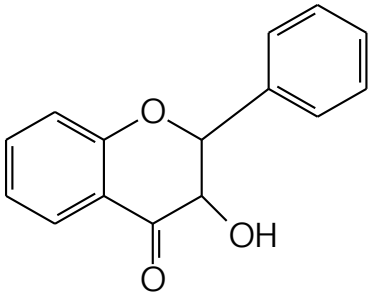
-Flavonol



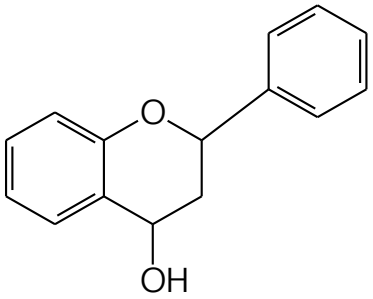
-Dihydroflavol



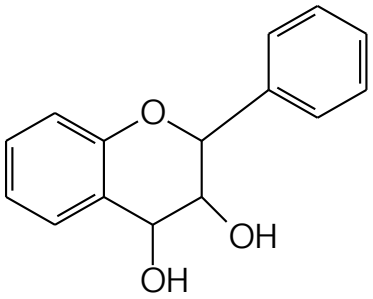
-Flavan-3-ol



-Flavan-4-ol



-Flavan-3,4-diol



Flavonoids are large group of phenolic plant constituents. To date almost 6500 different flavonoids have been identified. Flavonoids are mainly present in plants as O- or C-glycosides. Aglycones (the forms lacking sugar moieties) occur less frequently. At least 8 different monosaccharides or combinations of these (di- or

trisaccharides) can bind to the different hydroxyl groups of the flavonoid aglycones. The occurrence of a large number of flavonoids is the result of the many different combinations of flavonoid aglycones and these sugars. The most common sugar moieties include d-glucose, L-rhamnose, galactose and arabinose. The glycosides are usually O-glycosides, with the sugar moiety bound to the hydroxyl group at the C-3 or C-7 position, whereas the Cglycosides have sugar groups bound to a carbon (usually 6-C or 8-C) of the aglycone.

Flavonoids are divided into several subgroups. The most frequently encountere classification of flavonoid aglycones includes flavones, flavonols, flavanones, flavanonols,isoflavones, anthocyanidins, chalcones, catechins, and biflavonoids.

Flavonoid aglycones possess the chemical properties of phenolics, and thus they are slightly acidic. Those possessing a number of unsubstituted hydroxyl groups or sugar moieties are polar substances and soluble in polar organic solvents (e.g. water, ethanol). The presence of sugar makes flavonoids more water soluble.The intake of foods containing flavonoids reduces the risk of cancer. They are responsible for numerous biological activities including inhibition of cell growth, inhibition of protein kinase activity, inhibition of apoptosis, inhibition of MMP secretion, inhibition of tumor cell invasion, and inhibition of adhesion and spreading of cells; flavonoids also have anti-angiogenic properties. Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants. Since it was observed, that production of secondary metabolites is generally higher in differentiated plant tissues, there were attempts to cultivate whole plant organs, i.e. shoots or roots under in vitro conditions with the aim to produce medicinally important compounds. Flavonoids are produced by using different

biotechnological approaches, such as callus cultures, cell suspension cultures and/or organ culture [10].

1.4.4.1 Medical uses of flavonoids:

- Act as anti-(viruses cancers, adenitises and bacteria).

- Antioxidant.

- Alleviating pain, bruises and tumors.

- Work with vitamin “g” to protect capillaries.

- Reduce levels of cholesterol.

- Protect from symptoms of asthma.

- Protect from hypertension and heart diseases [11].

1.5 Solenostemmaargel:

1.4.1 Active ingredients present in S.argel:

Solenostemmaargel (Argel) known locally by the name (Hargel) .It is indigenous to Africa. The parts used are the leaves and stems; the leaves contain high carbohydrates and protein as well as crude oil, ash, calcium and magnesium. The leaves are used in herbal medicine for the treatment of some diseases such as of liver and kidney and allergies.It treats gastro-intestinal cramps, stomach-aches and urinary tract infections. Argel tea is used for lessening the pains of childbirth and for treating eating disorders since it increases appetite[12].

Solenostemmaargel, belongs to the Asclepiadaceae family. This family includes many wild growing medicinal plants. S.argel is considered to be medicinally important in the Sudan, Libya and Chad. Also, S.argel is a plant or plant part of valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body [13].

In addition, it was found that tissue cultures have produced compounds reviously undescribed and cultures of higher plant cells may provide an important source of new economically important compounds. Moreover, chemical investigations, chromatographic screening and phytochemical as well as tissue culture studies of S.argel leaves, stems and flowers revealed the presence of numerous biochemical ingredients such as pyrgene glycosides, flavonoids, kaempferol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids. In report on S.argel, showed the presence of kaempferol and steroidal glycosides in leaves of hargel also they found that the flavanoidscan be detected.solenostemmaargel contain flavonoids, kaempferol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids in S.argel. Also they contain pregnane ester glycosides in S.argel extracts. S.argel was found to include some flavanoidssaponins alkaloids. Moreover there are 2000 flavonoid found in S.argel found in asmethoxil or hydroxile group, further studies were needed to investigate this flavonoid. S.argel can be used medically in kidney disease, liver, respiratory system. Leaves of S.argel can be used an anti-inflammatory, antiseptic, vasodilatory and hypotensive.

Phytochemical studies of the leaves, stems and flowers revealed the presence of -amyrin and -sitosterol, 7-methoxy-3-22-dihydroxy-stigmastene, ethoxy derivative of vangurolic acid, an unidentified sterol. Moreover, they detected the presence of flavonoids and saponins in the different organs and alkaloids and/or nitrogenous bases in the leaves, stems and flowers. It containedacylated phenolic glycosides.

Alsoisolated -amyrin, sitosterol-containing rutin and quercetin from S.argel. S.argel. Solenostemmaargel contains an acidic resin, glycoside, choline,phytosterols and amyrins[14].

1.5.2 Traditional medicinal uses of S.argel:

Some uses Solenostemmaargel in folkloric medicine as treatment of GIT (Gastro Intestinal Tract) disturbances, hypercholesterolemia and diabetes mellitus; and externally in poultice form as anti-inflammatory and anti-rheumatic and inhalation of its smoke for the treatment of measles and cold. Moreover, „Hargal" infusion is used to treat jaundice, urinary tract infection and the disturbance of the menstrual cycle[15].

It is also used to cure stomach ache, anti-colic, remedy for suppurating wounds and anti-syphilitic when used for prolonged period of 40 to 80 days[16]. Alsoanti-inflammatory and ant rheumatic agent [17]. Again leaves are used as an antispasmodic, carminative and as an anti-diabetic[18].

In addition, it is used in indigenous medicine as an effective remedy for cough. The infusion of its leaves is used for gastro-intestinal cramps and infections of the urinary tract[19]. It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica[20].

The pharmacological activities of S.argel, including spasmolytic and uterine relaxant activities. It was [21] found that pregnane glycosides isolated from this plant were reported to reduce cell proliferation. Also the plant has antimicrobial activity[22]. The ethanol extracts of "Hargel" plant illustrated the presence of antibiotic substances. Similarly it was reported to have antimicrobial properties as well as antibacterial and antioxidant activity[23]. Moreover many studies

confirmed that the S.argel had remedial effect against numerous diseases and health problems such as diabetes mellitus [24] and cancer [25].

1.5.3 Toxicity:

S.argel had incurred hepatorenal toxicity in the experimental animals. Also in a feeding test with chicken a diet containing 10 leaves of solemenstommaargel caused a depression in growth and hepatotoxicity. The human use of S.argel, it could be of significance to propose for those seeking S.argel for treatment, to use the plant with the dose far below 600 mg/kg and to monitor closely the levels of creatinine, urea, alkaline phosphatase (ALP) and aspartate aminotransferase (AST) during the course of treatment.

On the other hand, it was found that the different types (leaves, extracts or alkaloids) of Solenostemmaargel tablets showed a very good therapeutic effectiveness (71%-100%) and a great margin of safety (98%-100%). No side effects or adverse reactions were recorded and the patients did not complain of any undesirable or intolerable toxic or adverse effects of these preparations of Solenostemmaargel [26].

1.6 Paper Chromatography:

A small spot of the compound or mixture is placed at the bottom of a strip of absorbent paper (filter paper). This end of the strip is dipped into a solvent , which is allowed to rise up the paper strip by the action of capillarity. If a single pure compound is applied to the paper , it moves from the origin , usually lagging behind the advancing solvent front , and forms a single spot at some point on the paper . If a mixture is applied to the paper , a series of spots usually results , corresponding to the components of the mixture , separation is effected to some

degree because each compound is differently partitioned between the developing solvent and the water phase that is present in paper under ordinary laboratory conditions. This is called paper-partition chromatography [27] .

1.7 Literature review:

Recent study was conducted to investigate the influence of soil applications of argel (Solenostemmaargel) dry leaves on flowering and yield of the dry date Barakawi cultivar in a completely randomized block design. Argel treatments were 0, 37.5 g once (1X1), 37.5 g twice (1X2), 37.5 g thrice (1X3), 75 g once (2X1) and 112.5 g once (3X1). Each treatment was replicated 5 times in two successive seasons under the conditions of the Northern State, Sudan. Argel treatments enhanced flowering and yield parameters of date palms and improved the physical characteristics of the fruits. As a nutritional role seems invalid due to the low amounts used, gains from argel leaves additives might owe to either a pesticide or a growth regulator-like effect [28]

Effect of Argel Leaf Aqueous Extract (Solenostemma argel) on Enzyme Activity in Gills and Muscles of Juvenile Nile Tilapia (Oreochromisniloticus) was also been reported[29]

The rodenticidaleffect of Argel leaves was studied under laboratory crude plant extract solved by ethanol and water or powder conditions. The free choice feeding test ,the crude ethanol ,water extract and dry powder leaves admixed will crushed maize to introduce it as a bait to the target animals, in different concentrations, were tested to clearify their rodenticidal activity against R. norvegicus(Albino). The results proved that the ethanol extract baits and dry powder baits were more effective than water extract baits[30]. Another study aimed to compare the anti-hyperglycemic effect of Solenostemma argel, which widely used for the treatment

of diabetes mellitus in Sudan, with the antidiabetic drug (Glibenclamide). Twenty four albino rats were used in this experiment. Rats were assigned to 4 groups (N=6). All groups were fasted for 18 hrs. Group (1) was administered with glibenclamide (10 mg/kg b.w.) and served as control, groups (2, 3, and) were orally administered with aqueous extract of Solenostemma argel leaves and bark (200, 400, and 800 mg/kg b.w.), respectively, after loading with 5% glucose (2 mg/kg b.w). Blood samples were obtained to assess blood glucose, lipid profile and α-amylase concentrations. Sub chronic toxicity of Solenostemma argel has been evaluated which clearly demonstrated the non-toxic nature and safety profile. Obtained results indicated that Solenostemma argel aqueous extract significantly decreased blood glucose level in treated group received 800 mg/kg b.w. compared with glibenclamide treated group. At the dose of 200 mg/kg b.w. of Solenostemma argel aqueous extract, the activity of α-amylase decreased in comparison with that treated with glibenclamide and registered low concentrations of cholesterol and HDL as well. In conclusion, both blood glucose level and α-amylase activity can be ameliorated in diabetic rats by administration of Solenostemma argel aqueous extract. However, in prospective study more investigation has should to be carried out to explain the mechanism of Solenostemma argel in hypoglycemic animals[31].

Objectives of the present study:

The aim of this study can be summarizes at the following points:

-Phytochemical screening to S.argel.

-Extraction of flavonoid.

-Separation of flavonoid.

-Characteristic of the obtained extract.

Chapter two

Materials and Methods

Chapter two

Materials and Methods

2.1Materials:

All chemical used in this research were of analytical grade type.

- Iodine (I_2).

- Distill water.

- Potassium iodide (KI).

- Mercuric chloride ($HgCl_2$).

- Bismuth Nitrate (Bi $(NO_3)_3$).

- Sodium hydroxide (NaOH) (10%).

-Lead acetate (Pb $(CH_3COO)_2$).

- Hydrochloric acid (HCl) (dilute).

- Copper acetate.($Cu(CH_3COO)_2$).

- Ethanol (CH_3CH_2OH) (99%).

- Methanol (CH_3OH) (99%).

- Beutanol (C_4H_9OH).

- Chloroform ($CHCl_3$) (99.5%).

- Acetic acid (CH_3COOH) (96%).

2.2Apparatuses:

- IR Spectrometer (IR Spectrometer 300 , Thermo Nicolet , Made in USA).

2.3Method:

2.3.1 Preparation of sample:

"Hargel" was collected from local market , and was dried in shadow.

2.3.2 Preparation of sample for phytochemical screening:

The dried "Hargel" was boiled in water for 30 min, and it was filtrated. Then the filtrate was taken for phytochemical screening.

2.3.3 Phytochemical screening methods:

Phytochemical examinations were carried out for all the extracts as per the standard methods.

2.3.4 Preparation of reagent:

2.3.4.1 Mayer's reagent:

3.5g of Mercuric Chlorided was dissolved in 60 ml of water and 5g of Potassium Iodide was dissolved in 10 ml of water , the solution of Mercuric Chloride was added to solution of Potassium Iodide then completed to 100 ml of water.

2.3.4.2 Wagner's reagent:

2g of iodine and 6g of potassium iodide was dissolved in 100 cm^3 of water.

2.3.4.3 Dragendroff's reagent:

3g of Bismuth Nitrate and 8g of Potassium Iodide was dissolved in 100ml of water.

2.3.4.4 Alkaline Reagent:

10 g of sodium hydroxide was dissolved in 100ml water.

2.3.4.5 Lead acetate reagent:

3g of lead acetate was dissolved in 10ml water.

2.3.4.6 Copper acetate reagent:

4g of copper acetate was dissolved in 12ml of water.

2.3.4.7 Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

2.3.4.8 Mayer's Test:

Filtrates were treated with Mayer's reagent .Formation of a yellow colored precipitate indicates the presence of alkaloid.

2.3.4.9 Wagner's Test:

Filtrates were treated with Wagener's reagent. Formation of brown/reddish precipitate indicates the presence of alkaloid.

2.3.4.10 Dragendroff's Test:

Filtrates were treated with dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloids.

2.3.5 Detection of flavonoids:

2.3.5.1 Alkaline Reagent Test:

Extracts were treated with few drops of sodium hydroxide 10%. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

2.3.5.2 Lead acetate Test:

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence offlavonoids.

2.3.6 Detection of diterpenes:

2.3.6.1 Copper acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

2.3.7 Extraction of flavonoid from the Solenostemmaargel:

Air dried Solenostemmaargel (80g) were powdered and extracted using 95% Ethanol at room temperature for 6 days. The extract was filtered,then the solvent was removed under reduced pressure at relativity low temperature.

2.3.8 Separation of flavonoid by paper chromatography:

Flavonoid was separated using paper chromatography by the solvents Chloroform and Methanol (2:1).

2.3.9 IR method:

One drop of the sample was placed in Kbr dish then pressed by another dish until it become a flat surface between the disk, the cell was placed in the IR device.

2.3.10 Testing of antibacterial susceptibility:

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.

Chapter Three

Results and Dissection

Chapter three

Results and Dissection

3.1 Results:

3.1.1 Phytochemical screening:

The following table show the result of alkaloids (Table 1).

Test	**Color**	**Result**	**Deducing**
Mayer's Test	Yellow color precipitate	+ive	Indicates the presence of al kaloid.
Wagner's Test	brown/reddish precipitate	+ive	Indicates the presence of alkaloid.
Dragendroff's Test	red precipitate	+ive	Indicates the presence of alkaloid.

The following table shows the result of flavonoid (Table 2).

Test	**Color**	**Result**	**Deducing**
Alkaline Reagent Test	yellow color	+ive	Indicates the presence of flavonoids.
Lead acetate Test	yellow color precipitate	+ive	Indicates the presence of flavonoids.

The following table shows the result of Diterpenes (Table 3).

Test	**Color**	**Result**	**Deducing**
Copper acetate Test	emerald green color	+ive	Indicates the presence of diterpenes.

3.1.2 Extraction of Flavonoids:

The extracted Flavonoid was a thick dark green crude and was about 20 g.

3.1.3 Separation of Flavonoid by Paper Chromatography :

The Flavonoids were separated by Paper chromatography into two Flavonoids green and yellow.

Figure (3-1): Separation of Flavonoid by Paper Chromatography

3.1.4 Analysis by IR :

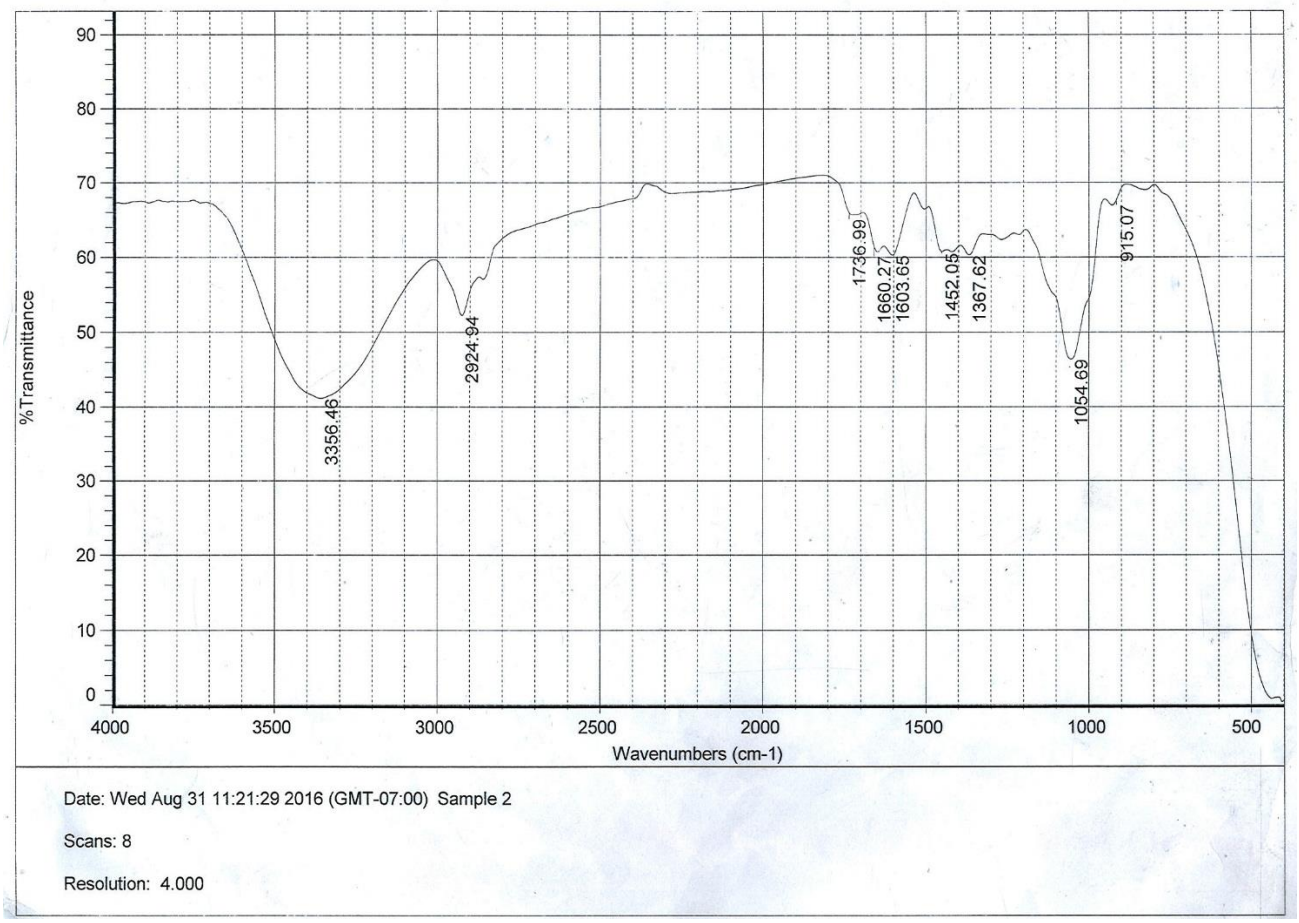


Figure (3-2): Shows IR Spectrum of Flavonoid (Yellow)

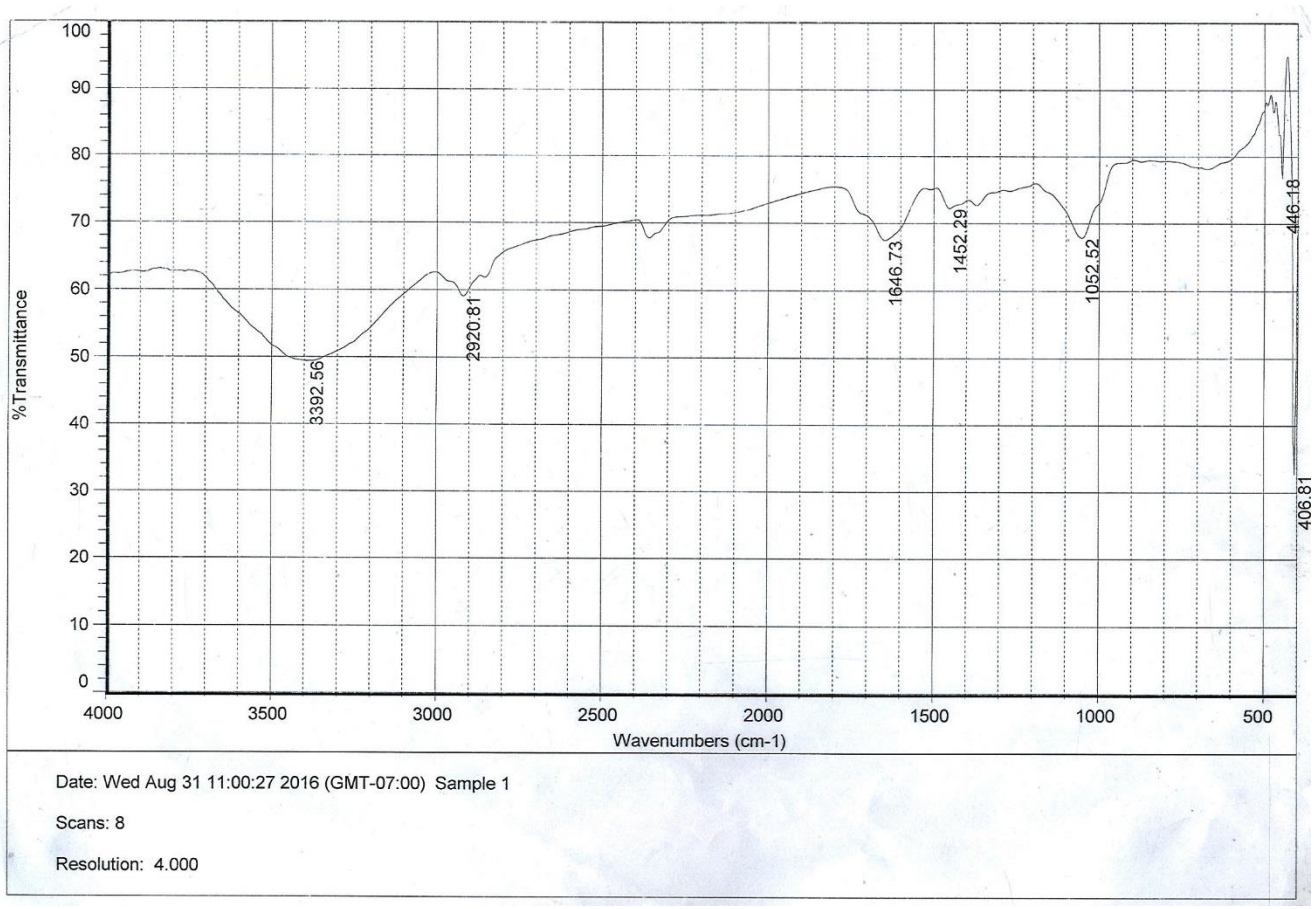


Figure (3-3): Shows IR Spectrum of Flavonoid (Green)

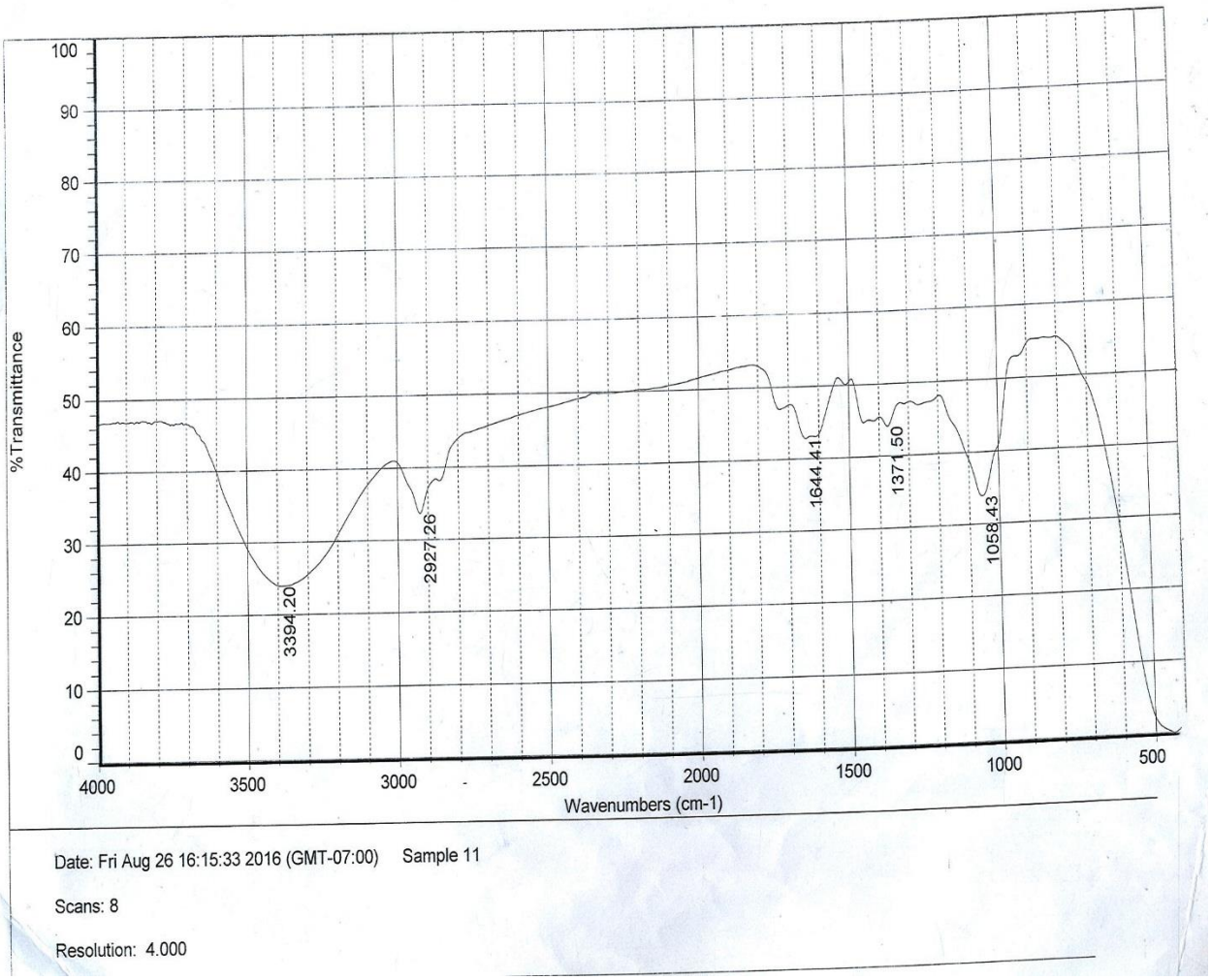


Figure (3-4): Shows IR Spectrum of Extract

3.1.5 Anti Bacteria :

The following table show the result of anti-bacteria in the extracted Flavonoid (Table 4)

Extract	Antibacterial Activity				
Flavonoid	Conc	Escherichia Coli	Pserdomonas aeruginosa	Staphylococcus aureus	Bacillus subtilis
	100	16	15	15	20
	50	15	14	13	19
	25	14	13	12	19
	2.5	13	13	10	18
	0.25	-	10	9	17

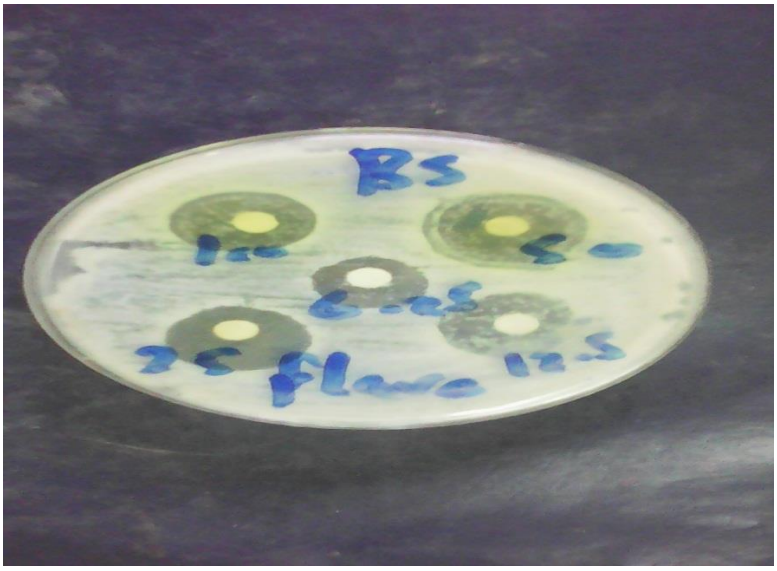


Figure (3-5)*: Bacillus subtilis*

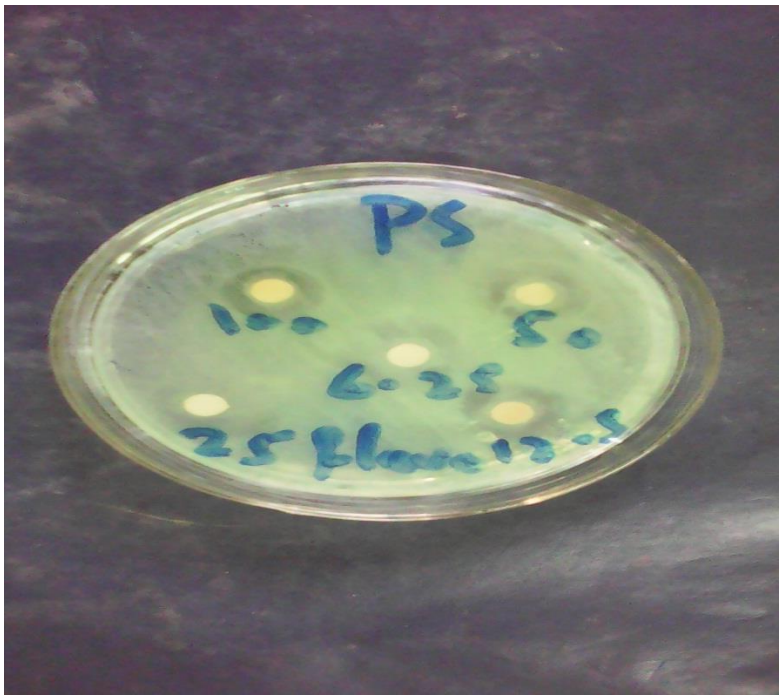


Figure (3-6): Pserdomonas aeruginosa

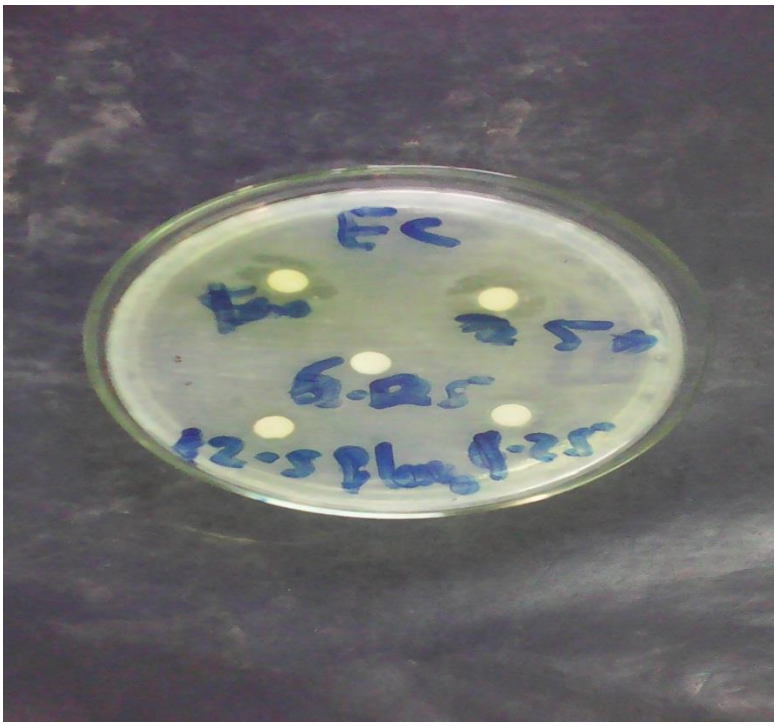


Figure (3-7): Escherichia Coli

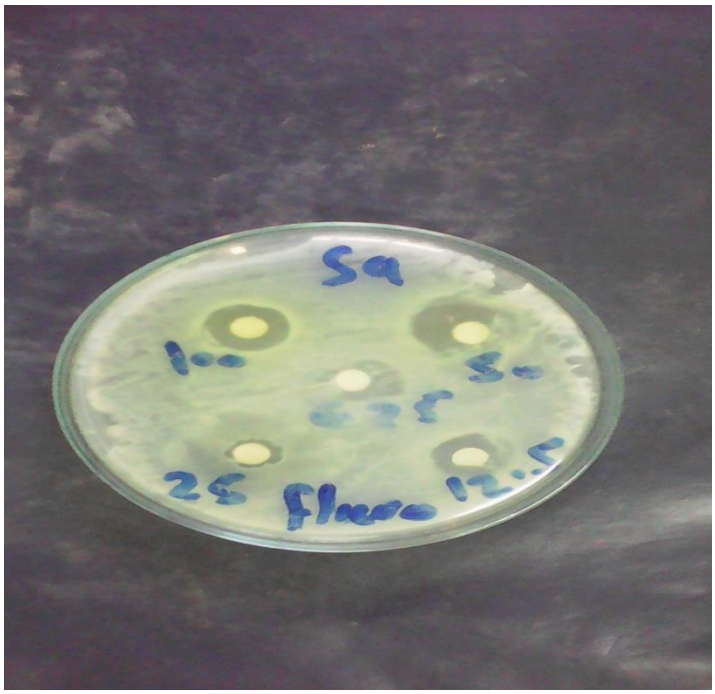


Figure (3-8): Staphylococcus aureus

3.2 Discussion:

Stems and leaves of S. argel were collected from North of sudan (Dongola, “agja”), then were boiled with water. The crude extracts were photochemical tested, a few amounts of alkaloids, flavonoids andDiterpenes were found (Tables 1, 2, 3).

Flavonoidswere the most obvious result in the photochemical test and it have the most beneficial medical uses so that why we extracted the Flavonoids using Absolute Ethanol, and after the extraction it were separated into two by Paper chromatography. The result we get is similar to a previous study but the difference that they use Thin Layer Chromatography and we used Paper Chromatography

Then the functional group was identified by IR spectrophotometer

The IR spectrum of the Flavonoid show peaks at 3394 (OH hydrogen bonding), 2927.26 (C-H aliphatic) , 1058(C-O) .

IR spectrum of the Green Flavonoid show peaks at 2920 (C-H) , 1052.52 (C-O)

The IR spectrum of the Yellow flavonoid show peaks at 1054.69 (O), 1660.27 (C=O) .

The result of the Antibacterial activity was positive and we noticed that the higher concentration has more activity, Escherichia Coli bacteriawhen it`s concentration was 0.25 the bacteria gave negative result

3.3 Conclusion:

In this study phytochemical screening of the S.Argel and extraction of flavonoids using Ethanol followed by paper chromatography separation have been ivistigated .The result obtained showed that Solenostemma argel contains different medicinal species such as flavonoids, alkaloids and diterpenoids. It also showed that the flavonoid had antibacterial activity. IR analysis indicated that all the characteristic peaks of flavonoid were appeared.

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