Sudan University of Science and Technology

College of Science

Department of Chemistry

Phytochemical Screening of the Bark of acacia polycanthia (Kakmot) and Isolation Tannin from it

A thesis Submitted in partial fulfillment for the Requirement of the degree of Bachelor

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الآيـــــــة

قَالَ تَعَالَىٰ:

﴿ أَفَرَءَيْتُمُ ٱلنَّارَ ٱلَّتِى تُورُونَ ﴿٧١﴾ ءَأَنتُمْ أَنشَأْتُمْ شَجَرَتَهَآ أَمْ نَحْنُ ٱلْمُنشِـُٔونَ ﴿٧٢﴾ ﴾

صدق الله العظيم

الواقعة: ٧١ - ٧٢

Dedication:

To our respective parents

To our family

Acknowledgement

First of all, we would like to thank Allah for given us strength, blessing and courage during this study and during all of our life,,

Special thank go to our supervisor Dr: Mohammed Sulieman Ali for his generous guidance and encouragement. His critical reading and questioning of our work has been a stimulus during field work planning thesis writing. We are grateful to him.

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Last but not least, Sudan University for Science and Technology for giving us the opportunity of holding B.Sc (Honor degree)

Above all, Allah for wonderful love and blessings in our achievements,,,

Abstract

The objective of this research is study the phytochemical screen to the bark of Accia polycanthia kakamout tree to know it is chemical composition and extract the active material by using maceration way and using the IR spectrum technique to detect the main composition of tannin which extracted from the bark of kakamout tree.

The result obtained the phytovhemical Screening indicated the presence of the percentage of tannin extracted was found to be 0.436%wt/wt.

After that IR measurements were applied to know the main function of the groups of tannin.

Also the anti bacterial activity was studied and the result reached to found to be a ppositive result from gram bacteria.

المستخلص

هدفت هذه الدراسه لإجراء المسح الكيميائي للحاء شجرة الكاكموت ومعرفة التركيب الكيميائي له ومن ثم تم إستخلاص التانين، بإستخدام طريقة الغمر في المذيب ، وأستخدمت الأشعة تحت الحمراء للتعرف علي المكونات الاساسيه للتانين ، وبعد ذلك أخضع لمضاد البكتيريا لمعرفة مدى فعاليته ضد البكتيريا، واوضحت النتائج ان كمية التانين المستخلص تساوي 0.436%(وزن/وزن).

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Short cut

Ps	Piper sarmentosum
Ca	Centellaasiaticn
Sa	Staphylococcus aurous
Ec	Escherichia coli

Chapter One

Introduction

1. Introduction:-

Definition of Natural Product:-

A natural product is a chemical compound or substance produced by a living organism—that is, found in[1,2] nature. In the broadest sense, natural products include any substance produced by life. Natural products can also be prepared by chemical synthesis (both semi synthesis and total synthesis) and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients.[3]

Within the field of organic chemistry, the definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism. Within the field of medicinal chemistry, the definition is often further restricted to secondary metabolites. Secondary metabolites are not essential for survival, but nevertheless provide organisms that produce them an evolutionary advantage. Many secondary metabolites are cytotoxic and have been selected and optimized through evolution for use as "chemical warfare" agents against prey, predators, and competing organisms.[2]

Natural products sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are the active components not only of most traditional medicines but also many modern medicines. Furthermore, because the structural diversity of

natural products exceeds that readily achievable by chemical synthesis, and synthetic analogs can be prepared with improved potency and safety, natural products are often used as starting points for drug discovery. In fact, natural products are the inspiration for approximately one half of U.S. Food and Drug Administration-approved drugs.[3]

1.2 Classification of natural product:

1.2.1 Stroide:

A steroid is an organic compound with four rings arranged in a specific configuration. Examples include the dietary lipid cholesterol, the sex hormones estradiol and testosteroneand the anti-inflammatory drug dexamethasone. Steroids have two principal biological functions: certain steroids (such as cholesterol) are important components of cell membranes which alter membrane fluidity, and many steroids are signaling molecules which activate steroid hormone receptors. [3]

The steroid core structure is composed of seventeen carbon atoms, bonded in four "fused" rings: three six-member cyclohexane rings (rings A, B and C in the first illustration) and one five-member cyclopentane ring (the D ring). Steroids vary by the functional groups attached to this four-ring core and by the oxidation state of the rings. Sterols are forms of steroids with a hydroxyl group at position three and a skeleton derived from cholestaneThey can also vary more markedly by changes to the ring structure (for example, ring scissions which produce secosteroids such as vitamin D_3). [4]

Hundreds of steroids are found in plants, animals and fungi. All steroids are manufactured in cells from the sterols lanosterol (animals and fungi) or cycloartenol (plants). Lanosterol and cycloartenol are derived from the cyclization of the triterpene squalene. [5]

1.2.2 Terpene:

Terpene are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, though also by some insects such as termites or swallowtail butterflies, which emit terpenes from their osmeteria. They often have a strong odor. They may protect the plants that produce them by deterring herbivores and by attracting predators and parasites of herbivores Many terpenes are aromatic hydrocarbons and thus may have had a protective function. The difference between terpenes and terpenoids is that terpenes are hydrocarbons, whereas terpenoids contain additional functional groups. [6]

They are the major components of resin, and of turpentine produced from resin. The name "terpene" is derived from the word "turpentine". In addition to their roles as end-products in many organisms, terpenes are major biosynthetic building blocks within nearly every living creature. Steroids, for example, are derivatives of the triterpene squalene. [6]

When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Some authors will use the term terpene to include all terpenoids. Terpenoids are also known as isoprenoids. [6]

Terpene and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as fragrances in perfumery, and in medicine and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives. Vitamin A is a terpene.

Terpene is released by trees more actively in warmer weather, acting as a natural form of cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature. (7)

The aroma and flavor of hops, highly desirable in some beers, comes from terpenes. Of the terpenes in hops myrcene, β-pinene, β-caryophyllene, and α-humulene are found in the largest quantities. (8)

Terpene are also major constituents of *Cannabis sativa* plants, which contain at least 120 identified compounds In addition to being responsible for the plant's aroma, they can act synergistically with cannabinoids. In fact, there are several promising applications based on the combined use of cannabinoids and terpenes, such as new acne therapies utilizing CBD with the monoterpenes limonene, linalool, and pinene; new antiseptic agents with CBG and pinene; treatment of social anxiety disorder using CBD with limonene and linalool; and treatment of sleeping disorders by adding caryophyllene, linalool, and myrcene to 1:1 CBD/THC extracts. (9)

1.2.3 Alkaloid:

Are a group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Some synthetic compounds of similar structure are also termed alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and, more rarely, other elements such as chlorine, bromine, and phosphorus. [10]

Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants, and animals. They can be purified from crude extracts of these organisms by acid-base extraction. Alkaloids have a wide range of pharmacological activities including ant malarial (e.g. quinine), antiasthma (e.g. ephedrine), anticancer (e.g. homoharringtonine), cholinomimetic (e.g. galantamine) vasodilator (e.g. vincamine), ant arrhythmic (e.g. guanidine), analgesic (e.g. morphine), antibacterial (e.g. chelerythrine), and ant hyperglycemic activities (e.g. piperine). Many have found use in traditional or modern medicine, or as starting points for drug discovery. Other alkaloids possess psychotropic (e.g. psilocin) and stimulant activities (e.g. cocaine, caffeine, nicotine, the bromine), and have been used in entheogenic rituals or as recreational drugs. Alkaloids can be toxic too (e.g. atropine, tubocurarine) although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly evoke a bitter taste. [11]

The boundary between alkaloids and other nitrogen-containing natural compounds is not clear-cut. Compounds like amino acid peptides, proteins, nucleotides, nucleic acid, amines, and antibiotics are usually not called alkaloids Natural compounds containing nitrogen in the exocyclic position

(mescaline, serotonin, dopamine, etc.) are usually classified as amines rather than as alkaloids. Some authors, however, consider alkaloids a special case of amines. (12)

1.2.4 Flavnoid:

Flavonoids (or bioflavonoid) (from the Latin word *flavus* meaning yellow, their color in nature) are a class of plant and fungus secondary metabolites.

Chemically, they have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C). This carbon structure can be abbreviated C6-C3-C6. According to the IUPAC nomenclature,[1][2] they can be classified into: (13)

- *flavonoids* or *bioflavonoid*
- *isoflavonoids*, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure
- *neoflavonoids*, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure

The three flavonoids classes above are all ketone-containing compounds, and as such, are anthoxanthins (flavones and flavonols). This class was the first to be termed *bioflavonoid*. The terms *flavonoids* and *bioflavonoid* have also been more loosely used to describe non-ketone polyhydroxy polyphenol compounds which are more specifically termed flavonoids. The three cycles or heterocyclic in the flavonoids backbone are generally called ring A, B and C. Ring A usually shows a Phloroglucinol substitution pattern. (13)

1.2.5 Tannins:

1.2.5.1 Definition:

A tannin (or tannoid) is an astringent, polyphenol bimolecular that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids.

The term tannin (from *tanna*, an Old High German word for oak or fir tree, as in Tannenbaum) refers to the use of wood tannins from oak in tanning animal hides into leather; hence the words "tan" and "tanning" for the treatment of leather. However, the term "tannin" by extension is widely applied to any large polyphenol compound containing sufficient hydroxyls and other suitable groups (such as carboxyl) to form strong complexes with various macromolecules.

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine or tea. Likewise, the destruction or modification of tannins with time plays an important role when determining harvesting times. [14]

Tannins have molecular weights ranging from 500 to over 3,000 (gallic acid esters) and up to 20,000 (proanthocyanidins). [15]

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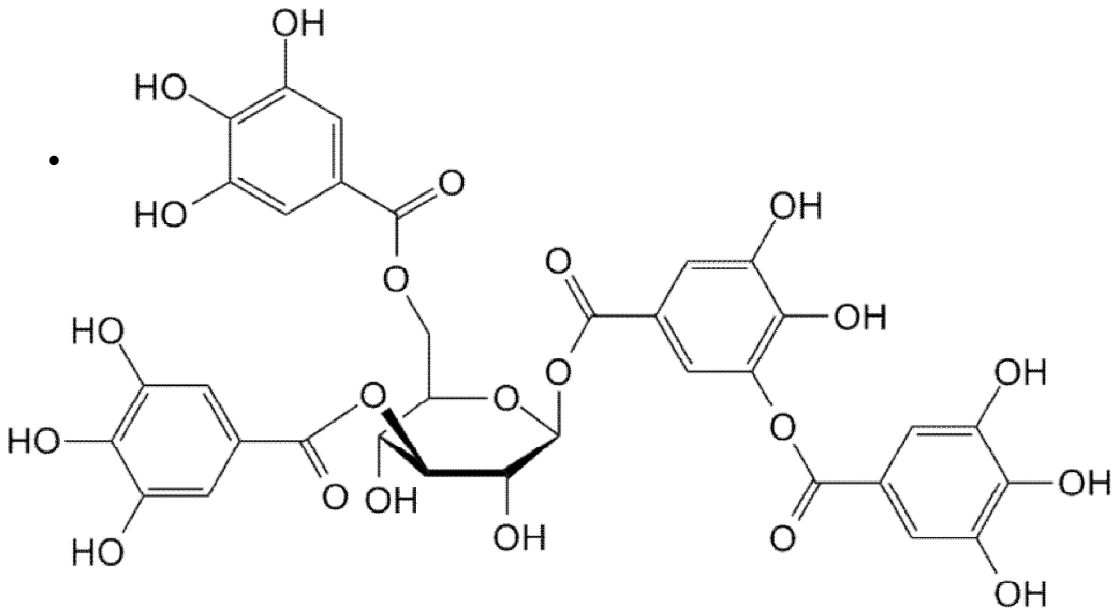
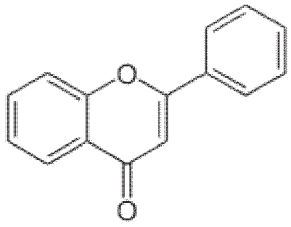
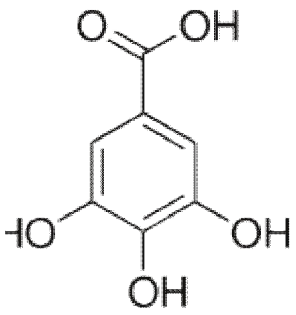


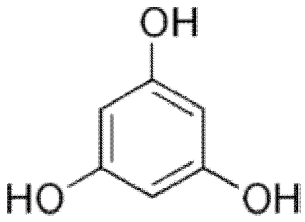
1.2.5.2 Structure and classes of tannins:

There are three major classes of tannins Shown below are the base unit or monomer of the tannin. Particularly in the flavone-derived tannins, the base shown must be (additionally) heavily hydroxylated and polymerized in order to give the high molecular weight polyphenol motif that characterizes tannins. Typically, tannin molecules require at least 12 hydroxyl groups and at least five phenyl groups to function as protein binders.



Flavone





Phloroglucinol

Base Unit:	Gallic acid		
Class/Polymer:	Hydrolyzable tannins	Non-Hydrolyzable or condensed tannins	Phlorotannins
Sources	Plants	Plants	Brown algae

Oligostilbenoids (oligo- or polystilbenes) are oligomeric forms of stilbenoids and constitute a class of tannins. [16]

Pseudo tannins:

Pseudo tannins are low molecular weight compounds associated with other compounds. They do not change color during the Goldbeater's skin test, unlike hydrolysable and condensed tannins, and cannot be used as tanning compounds Some examples of pseudo tannins and their sources are: [17]

Pseudo tannin	Source(s)
Gallic acid	Rhubarb
Flavan-3-ols (*Catechins*)	Tea, acacia, catechu, cocoa, guarana
Chlorogenic acid	Nux-vomica, coffee, mate
Ipecacuanhic acid	*Carapichea ipecacuanha*

1.2.5.3 Tests for tannins:

There are three groups of methods for the analysis of tannins: precipitation of proteins or alkaloids, reaction with phenolic rings, and depolymerization.[18]

- *Goldbeater's skin test*

When goldbeater's skin or ox skin is dipped in HCl, rinsed in water, soaked in the tannin solution for 5 minutes, washed in water, and then treated with 1% $FeSO_4$ solution, it gives a blue black color if tannin was present.

- *Ferric chloride ($FeCl_3$) test*

It is rather a test for phenolic in general. Powdered plant leaves of the test plant (1.0 g) are weighed into a beaker and 10 ml of distilled water are added. The mixture is boiled for five minutes. Two drops of 5% $FeCl_3$ are then added. Production of a greenish precipitate was an indication of the presence of tannins. Alternatively, a portion of the water extract is diluted with distilled water in a ratio of 1:4 and few drops of 10% ferric chloride solution is added. A blue or green color indicates the presence of tannins (Evans, 1989). [19]

- *Other methods*

The hide-powder method is used in tannin analysis for leather tannin and the Stiasny method for wood adhesives. Statistical analysis reveals that there is no significant relationship between the results from the hide-powder and the Stiasny methods. [20]

- *hide-powder method*

400 mg of sample tannins are dissolved in 100 ml of distilled water. 3 g of slightly chromate hide-powder previously dried in vacuum for 24h over $CaCl_2$ are added and the mixture stirred for 1 h at ambient temperature. The suspension is filtered without vacuum through a sintered glass filter. The weight gain of the hide-powder expressed as a percentage of the weight of the starting material is equated to the percentage of tannin in the sample.

- *Stiasny's method*

100 mg of sample tannins are dissolved in 10 ml distilled water. 1 ml of 10M HCl and 2 ml of 37% formaldehyde are added and the mixture heated under reflux for 30 min. The reaction mixture is filtered while hot through a sintered glass filter. The precipitate is washed with hot water (5x 10 ml) and dried over $CaCl_2$. The yield of tannin is expressed as a percentage of the weight of the starting material.

- Reaction with phenolic rings

The bark tannins of Commiphora angolensis have been revealed by the usual color and precipitation reactions and by quantitative determination by the methods of Löwenthal-Procter and of Deijs (formalin-hydrochloric acid method). Colorimetric methods have existed such as the Neubauer-Löwenthal method which uses potassium permanganate as an oxidizing agent and indigo sulfate as an indicator, originally proposed by Löwenthal in 1877. The difficulty is that the establishing of a titer for tannin is not always

convenient since it is extremely difficult to obtain the pure tannin. Neubauer proposed to remove this difficulty by establishing the titer not with regard to the tannin but with regard to crystallized oxalic acid, whereby he found that 83 g oxalic acid correspond to 41.20 g tannin. Löwenthal's method has been criticized. For instance, the amount of indigo used is not sufficient to retard noticeably the oxidation of the non-tannins substances. The results obtained by this method are therefore only comparative. A modified method, proposed in 1903 for the quantification of tannins in wine, Feldman's method, is making use of calcium hypochlorite, instead of potassium permanganate, and indigo sulfa. [20]

1.3 Medicinal Plant Constituents:- [21]

The natural of the active constituents or constituents of a crude drug obtained from a medicinal plant determination its pharmacological action or its therapeutic activity. The active constituent may be either a single chemical substance e.g glycoside, alkaloid, or a mixture of substance e.g fixed oils , volatile oils , resins , etc. The separation of the mixture is neither practical nor advantageous.

The active constituent is often used is preference to the drug itself or a preparation of the drug, especially when it is a commercial proposition to isolate and purify it or to synthesis it on large scale.

This has certain advantages : medically it can guarantee a more exact dosage , pharmaceutically it can lead to a more suitable or elegant preparation free from undesirable inter constituents , and economically it usually ensures a regular supply at fairly uniform prices.

There are of course a number of example where the therapeutic effect of the medicinal plant or drug obtained from it , or galenical preparations to of the drug differ to some extent from that of its active constituents in isolation this may be due to a synergistic effect of several constituents present in the drug , as in case of senna leaf . It may be also due to a modifying effect by some constituents upon the physical properties of the others. Digitalis because of the solubilising effect of the saponin , digitonin .

When the constituents of the crude drug have uncertain tnerapeutic value ,it is economically unsound to isolate these constituents or synthesise them . The galenical forms are still prescribed even for those drugs which are gradually being replaced in medicine by the pure chemical substance . In order ensure that the galenical or the pharmaceutical preparation is therapeutically active and elegant i.e showing no precipitation or other chemical changes even after storage for sometime ; it is necessary to have a knowledge not only of the physiologically active constituents of a drug , butalso of the inter substance which might be present , particularly tannins , proteins and enzymes . Such knowledge would enable maximum isolation and suitable methods of purification of the constituents to be made , if such was thought desirable . It would also enable the production of galenicals which are elegant and , which truly represent the activity of the drug itself .

It follows , therefore , that a sound knowledge of plant chemistry , particularly as applied to drugs should be acquired . That is to say ,the medicinal plant or the drug derived from it ,which is known as a botanical entity , should be interpreted in chemical terms .

The knowledge of plant chemistry is also necessary for running micro-chemical tests required for identification of crude drugs and the localization of their constituents in the tissues . Such knowledge is also necessary in breeding experiments, for the selection and improvement of medicinal plants to obtain strains with higher yields of active constituent. This requires knowledge of genetics and biogenesis, as well as knowledge in the separation, purification, and assay of the different plant constituents to determine their percentage in the plant or plant organ or in the crude drug obtained from them .

The chemical examination of a medicinal plant or a vegetable product can be a very lengthy prcceeding , and generation of workers have but gradually elucidated the constituents of such drugs as ergot and digitalis .Work still continues even with some of the best known drugs . It is , therefore , very important that any worker in this phytochemistry field ,should make sure before the embarks on chemical work that he has , or can be sure of obtaining , adequate supplies of the raw material and that this is authenticated by botanical means .

It should be realized that the dried vegetable drug ,although a dead plant or a part of plant , was once a living organism .Many plant constituent are relative stable and there for occur both in the fresh plant and in dried drug ; other may undergo changes brought about by the enzymes , heat and moisture during drying and extraction processes . Sometimes these changes are very complex and condition must be strictly controlled to give the product desired . Familiar example of this are preparation of tea , coca and tobacco.

The living organism may be consider as abiosynthetic laboratory in which many metabolic processes take place , not only for chemical compounds(carbohydrates , proteins ,fats)that are utilized as food for man and , but also for a multitude of secondary compounds (glycosides , alkaloids , volatile oils), that extract a physiological effect .These chemical compounds give plant drugs their therapeutic properties.

The study of the biochemical pathways leading to the formation of secondary constituents used as drugs has been much increasing in recent years .In this respect , isotopically labeled organic compound (tracer substances)have been widely used .These satisfactory explanation have been advanced for the presence of these secondary constituents in great number of plant families .

Nevertheless, their occurrence in related genera and their chemical relationships indicate that they may be a factor in the basic metabolism of plants .

1.4 Phytochemical Classification:-

Many of they pharmacologically active naturally occurring compounds present in medicinal plants , have large molecules which often contain more than one of the chemical groupings which are characteristsic for alcohols , aldehydes , ketones ,phenols , esters and organic acid ,ect .these chemical grouping are attached to molecular skeletons of varying nature and omplexity.

It would , therefore , be unsatisfactory to consider in the same class , a simple substance ,such as salicylic acid and a complex one like morphine

,although both have a phenolic OH group in their molecule .On the other hand , some naturally occurring mixtures of substances such as volatile oils , are conveniently considered together ,although they may be mixture of hydrocarbons , aldehydes ,ketones ,ester… etc ,and in these even the skeleton varies in being sometimes a chain and sometimes one or more rings of atoms .

1.5 Chemical of Plant Constituents:-

1-Containing C and H only.

Hydrocarbons : ocimene , limonene , p-cymene .

2-Containing C ,H and *O*

- Alcohols:
- Aldehydes:
- Ketones:
- Phenols:
- Quinones:
- Acid:
- Esters:
- Lactones:
- Terpenoids:
- Carotenoids:
- Steroids:

3-Containing Oxygen in two heterocyclic rings:

- Derived from furan
- Derived from pyran

- Derived from flavan
- Derived from phenylbenzopyrilium
- Carbohydrate

4-Containing Elements Other than C ,H and O:

- Heterosides or glycosides
- Lipid

5-Always containing nitrogen

Amino acid ,peptides ,proteins and enzymes.

Amines and related nitrogen compounds ,trimethylamine ,capsaidine ,trigonelline

Alkaloids

6-Mixtures*:*

- Tannins
- Volatile oils
- Resins
- Latex

7-Classes of Varied Chemical Nature:

Vitamins . Antibiotics

Some of the chemical classes referred to above are minor interest as medicinal agente.other of greater importance such as volatile oils,resins glycosides and alkaloids.

1.6 acacia polycathia: [22]

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Fabales
Family:	Fabaceae
Genus:	*Senegalia*
Species:	*S. polyacantha*
Binomial name	
Senegalia (Willd.) Seigler & Ebinger	*polyacantha*
Subspecies	
• *Senegalia polyacantha* subsp. *campylacantha* (Hochst. ex. A.Rich.) Kyal. & Boatwr. • *Senegalia polyacantha* subsp. *polyacantha* (Willd.) Seigler & Ebinger	
Synonyms	
• *Acacia catechu sensu* Griseb. • *Acacia catechu auct. non* L. • *Acacia polyacantha* Willd. • *Acacia suma* (Roxb.) Voigt	

- *Gagnebina tamariscina sensu* Bojer
- *Mimosa suma* Roxb.
- *Senegalia suma* (Roxb.) Britton & Rose

Description:

Tree reaching 15 to 21 m in height. The trunk with fissured, ash-grey bark, with brown scales and black knots on the place of insertion of former leaves and thorns. Slash red-brown with white streaks. Knobbly persistent prickles in pairs below each node, straw coloured to brown or black, with a black tip, 4 to 12 mm long. Leaf petiole glandular up to 25 cm long, with 10-40 pairs of pinnae bearing 15-60 pairs of leaflets each. Leaflets 4-6 mm long and 0.5-1 mm wide. Petiole broadening to 5 mm at the bottom with a large gland between the second and third pairs of pinnulae, occasionaly with small, solitary spines. Flowers cream or white in spikes 6-12 cm long set in pairs or triplets. Pods brown, dehiscent, 7-18 cm long, 1-2 cm wide, flat, dark brown, containing 5-10 seeds that can be seen through the translucent wall. The tree has a good germination capacity and quick juvenile growth.

Soil:

Deep fresh soils medium textured to clayey, well drained, neutral to alkaline, occasionally on compacted hard soils and stony slopes.

Distribution:

Widespread in tropical Africa from the Gambia to Eritrea, Ethiopia, in the north, to the Transvaal in the south. A subspecies, polyacantha, occurs in India. A. polyacantha is suspected to have been introduced from the Indian sub-continent.in the olden days, and now completely naturalized. This is not the only such case, see Tamarindus indica.

In the Sudanian and Guinean savannas, restricted to well watered places in the South Sahel ecozone, around ponds and in the bottom of fossil valleys with a shallow water-table, but sensitive to water-logging. Not a gregarious nor very common species. A. polyacantha is a tree of the sub-humid to humid African tropics with a wide distribution from South Senegal to Ethiopia and East Africa.

Crop management:

In spite of its feeding quality, it is a most undesirable tree; has strong, pernicious, recurved spines, and spreads rapidly on fallow land in pasture, particularly on low-lying fertile alluvial soil along the drainage lines (in Zambia). It is expensive and difficult to eradicate. Large trees can be killed by ringbarking and the application of arboricides.

Products & uses:

Hard durable wood ; sapwood white, heartwood red with blackish streaks ; service wood, poles, posts, various tools, handles, wheels etc. easy to polish, but difficult to saw. Good fuel wood and charcoal, gum edible, heartwood chips are used for tanning and dyeing, ashes are a substitute for salt. Human

medicine : roots are an antidote to snakebite, bark decoction used for dysentery, to cure veneral diseases and gastro-intestinal disorders, a tonic beverage is made from the roots. Trees are pollarded for forage.

Uses:

Repellent uses:

The root of Senegalia polyacantha subsp. campylacantha emits chemical compounds that repel animals including rats, snakes and crocodiles.[2]

Gum:

The tree's gum is used in the manufacture of candy.

Medicinal purposes:

S. polycantha's roots and perhaps its bark have medicinal uses. The root extract is useful for snakebites[2] and is applied to wash the skin of children who are agitated at night time. The root is also used for treating gonorrhea venereal diseases, dysentery and gastrointestinal disorders.

Tannin:

The bark is useful for tanning.

Wood:

The tree's primary use is for wood

1.7 Objectives of the research:

To detect the active materiale in the bark of the acacia polycanthia;to extract it.

Chapter Tow

Chemicals and Methods

2-Chemical and method:-

2-1 Apparatus:

Funnel, dropper, test tube, beaker, filter paper, separator funnel, evaporating dish, hot place, 500ml Erlenmeyer flask.

2-2 Instruments:

Sensitive balance (AND, Max 120g Min 10mg, d = 0.1mg),

IR spectrometer (USA Berime company to scholarliness equipment IR 300 spectrometer)

2-3 Chemicals:

Sample powder, ferric chloride ($FeCL_3$ 5%), cocentreted sulfuric acid (H_2SO_4), hydrochloric acid (HCL) diethyl ether ($CH_3CH_2OCH_2CH_3$) acetone ($CH_3C{=}OCH_3$ 70%), distilled water.

2-4 Phytochemical screening:

2-4-1 Test of alkaloids:

The test solution was mixed with little amount of hydrochloric acid and Mayer's reagent, formation of white precipitate, to indicate of presence of alkaloids.

2-4-2 Test of tannins:

1.0g of sample powder was weighted into beaker and 10ml of distilled water was added, the mixture was boiled for five minutes, two drops of 5% ferric

chloride was added production of greenish precipitate was indicate the presence of tannin.

2-4-3 Test of flavonoides:

All flavanoids give yellow or orange color with sulfuric acid , 1.0 gram of powder sample was weighted, and 10ml of distilled water was added, the mixture was boiled for five minutes, 5ml of mixture transferred to test tube few drops of sulfuric acid was added give negative result. [24]

2-5 Procedure:

100 grams portioned of powder sample was weighted and subjected to reflux for 15 minutes in 500ml flask enough 70% acetone to cover up the whole powder sample the flask will be removed from the hot plate and will be filtered.

In another flask, sufficient amount of 70% acetone was added into the filter paper to wash of the filtered.

The washed solution will be combined to the filtered, the filtrate will be subjected to the extraction method using diethyl ether, and this will be done in five times, until complete separation of the diethyl ether and tannin, which was upper layer and lower layer respectively.

The tannin has to be collected using separator funnel; the collected tannin will be evaporated until the residue was obtained.

The residue will be final product and called the extracted tannin.

The percentage of tannin was calculated by using this formula:

Tannin % = $weight\ of\ tannin$ /weight of powder sample × 100. [24]

2-6 Characterization methods:

2-6-1 IR measurement:

FT-IR transmittance spectra of the separated components were obtained using a Shimadzu FT-IR spectrometer in the wave number range of 4000 to 500 cm^{-1} . The powdered sample was thoroughly mixed potassium bromide, pressed to make pellet, and then scanned to obtain its IR spectrum. [23]

2-6-2 Antibacterial activity:

The principle of the agar plate dilution is the inhibition of the growth on the surface of the agar by the plant extracts incorporated into the medium.

Plates were prepared in the series of decreasing concentrations of the plant extraction in the following order 100, 50, 25, 12.5, 6.25; 3.125 mg/ml. The bottom of each plate was marked off into 6 segments. The organisms tested were grown in broth over night to contain 10^8 organisms per ml. Loop-full of diluted culture is spotted with a standard loop that delivers 0.001 ml on the surface of each segment and then incubated at 37 ºC for 24 hours.

The end point (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth. Results are reported as the MIC in mg/ml.

Chapter three

Results and discussion

3- Results and discussion:-

3-1Results

3-1 Table (3.1) shows the result of phytochemical screening of acanthi poly acacia:

type of test	Test	Observe	Result
Alkaloids test	1ml of test solution+ few drops of HCl+ few drops of Mayer's reagent	No occurs change	Negative test of alkaloids
Tannin test	Test solution +few 5%)(Drops of $FeCl_3$	Formation greenish color	Positive test of tannins
Flavonoids test	Test solution +few drops of H_2SO_4	No occurs change	Negative test of flavonoids

3-2 table (3.2) shows percentage of tannin from barks of acanthi poly acacia (kakamout):

Weight of glass	Weight of glass and tannin	Weight of tannin
51.8334	52.2694	0.436

3-2 the percentage of tannin:

Tannin%= $\frac{\text{weight of tannin}}{weight\ of\ sample} \times 100$

$\frac{0.436}{100} \times 100 = 0.436$

3-3 Table (3.3) shows functional groups results of IR spectrum:

Functional groups	Absorbance
O-H	3408.15
C-H alkanet (stretch)	2925.34
C-H aldehayde	2856.89
C-H alkane (bend)	1455.17
C=O	1742.47
C-O	1071.47
C=C alkenes	1616.65
C=C aromatic	1514.24
C-H out of plane	666.40
C-X substitution	455.70

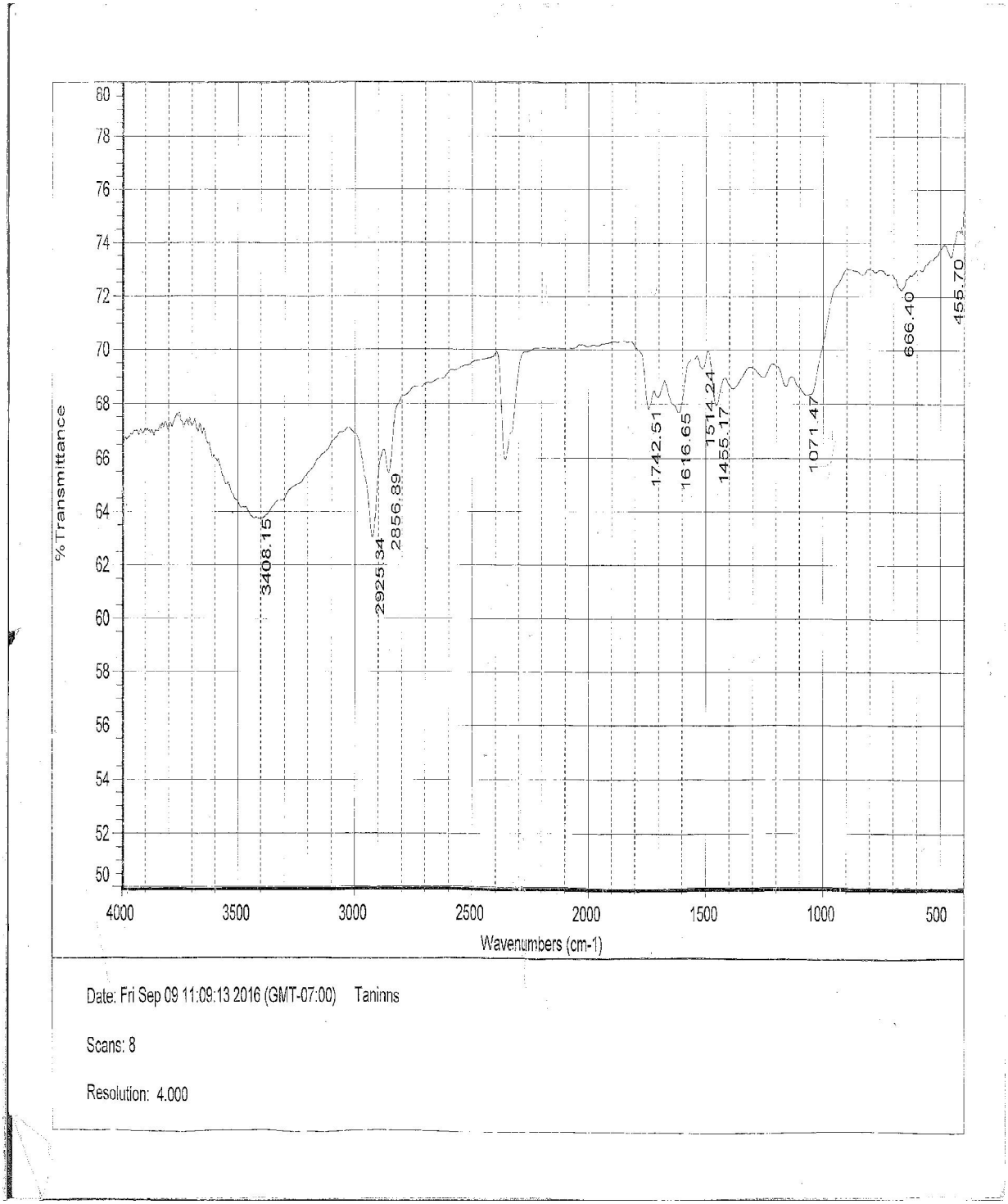


Figure (3.1) shows IR Spectrum of Tannin

3-3IR measurement:

FTIR analyses were carried out to confirm the presence of the functional groups of tannin which were reported to be the main constituent of the tannins .the figure3 represents the FTIR spectrum of tannins. The main functional groups of tannin are: O-H bond, C-H alkane (stretch),C-H aldehyde,C-H alkane bend, carbonyl group , C-O bond, C=C alkene , C=C aromatic, C-H out of plane , C-X substitution. Scan is seen from the spectrum. [23]

3-4 Tables (3.4) show antibacterial activity and antifungal activity:

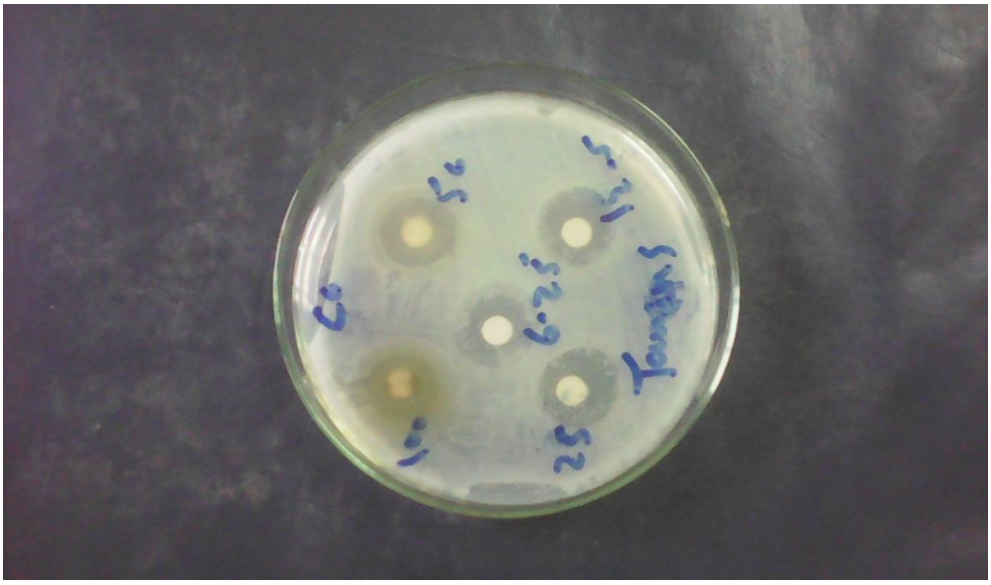
	Antibacterial activity					**Antifungal activity**
Extracts	Ec	Ps	Sa	Bs	Ca	
100mg/ml	21	21	17	-	17	
50mg/ml	20	20	16	-	16	Mm
25mg/ml	15	19	-	-	15	
12.5mg/ml	14	18	-	-	14	
6.25mg/ml	14	17	-	-	13	

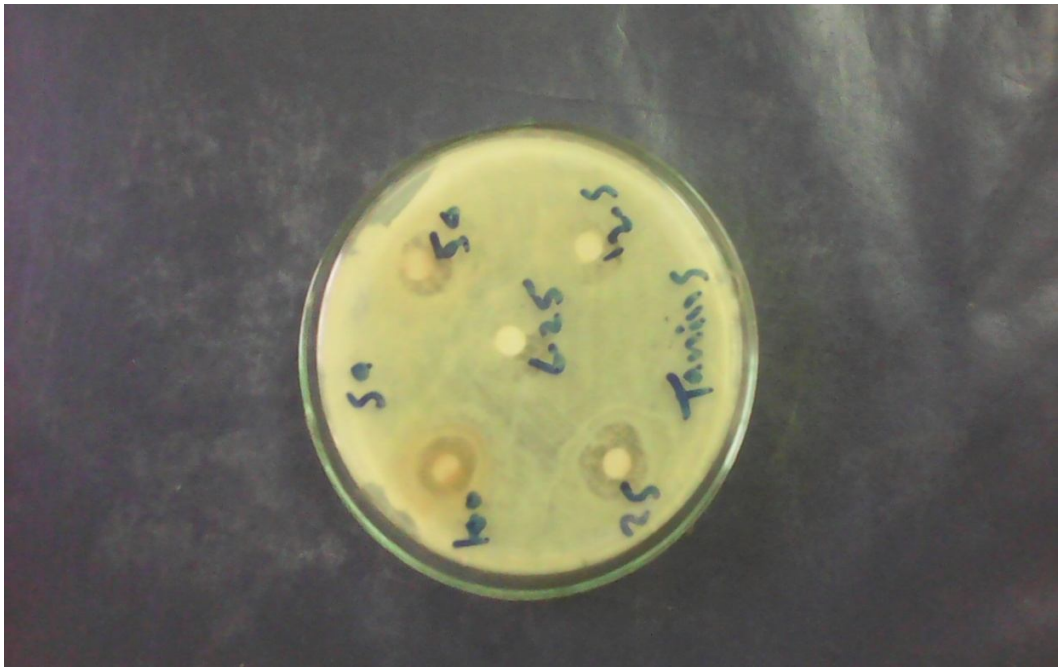
-v e (Ec and Ps) = gram negative anti bacteria

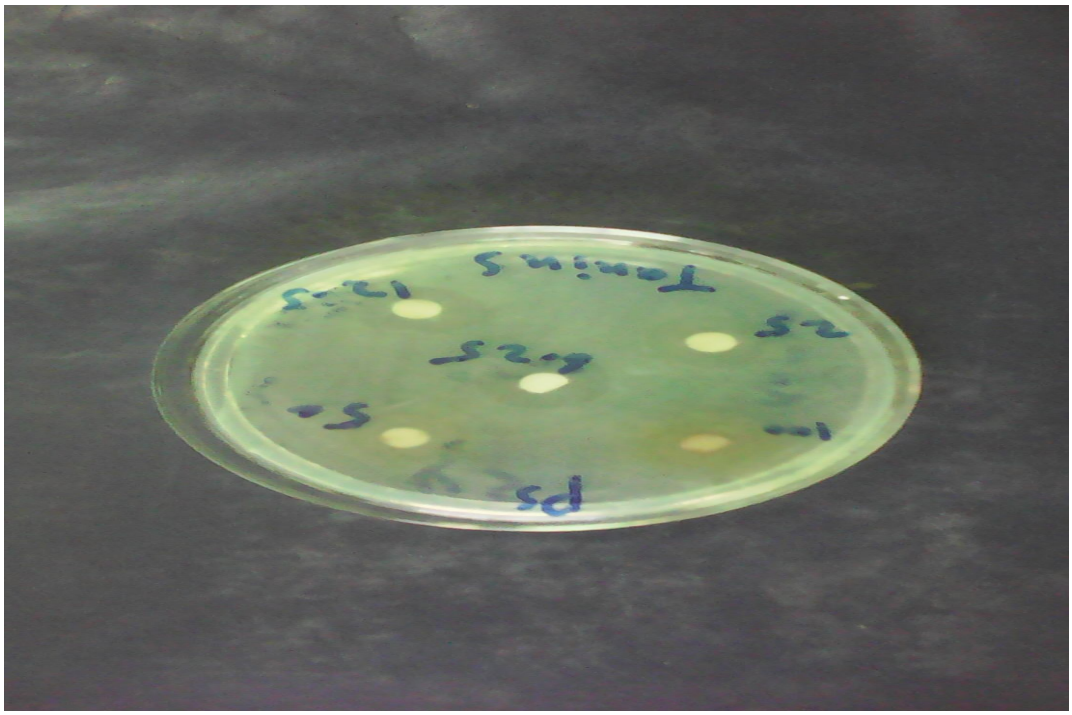
+v e (Sa and Bs) = gram positive anti bacteria

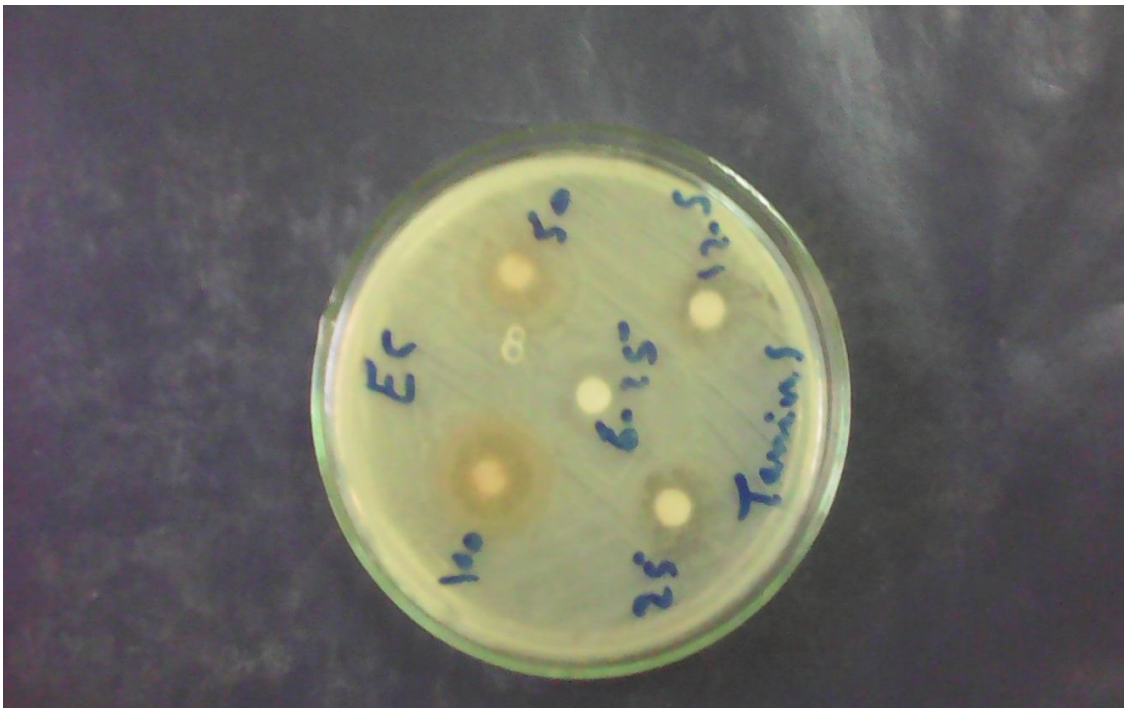
F (Ca) = fungi.

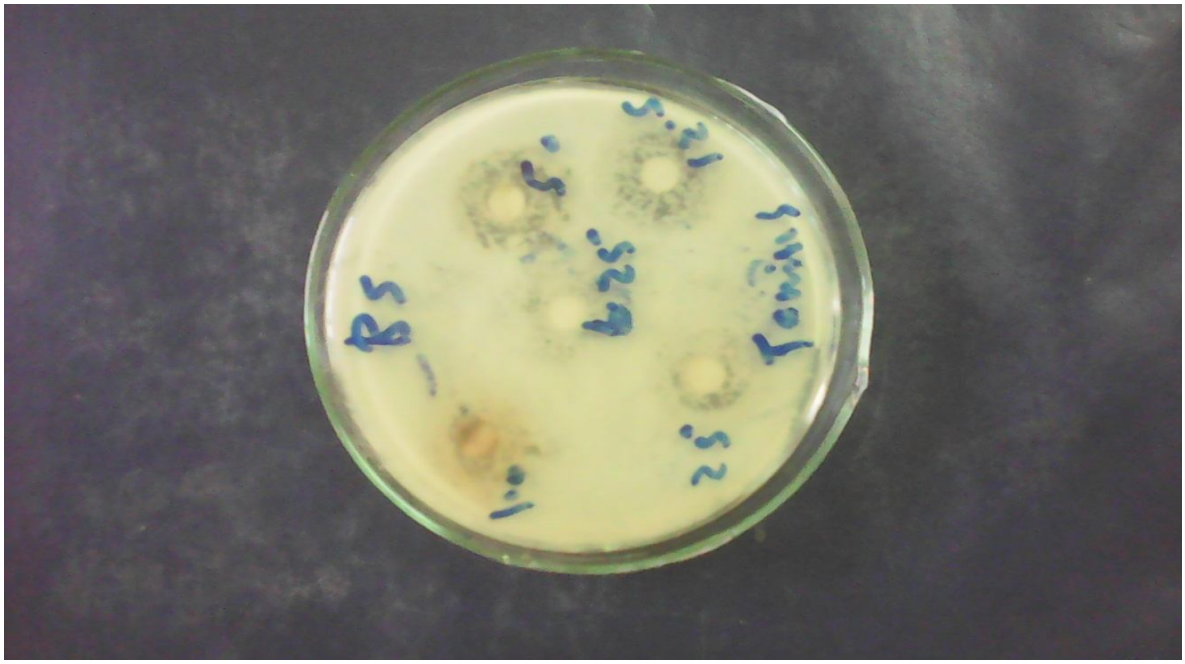
This is shows in the below figures:











Figures (3.2) show anti bacteria activity

3-4 Discussion:

The tannin was extracted from the barks of acanthi poly acacia by separator funnel and the percentage yield of it was found to be equal 0.436%.

After the extracted were applied infrared spectra upon the tannin to identify the component of it. the broad and intense peak in range between 3200 and 3600 cm^{-1} is due to the absorption of OH group. Strong sharp peak at 1742.51cm^{-1} due to absorption of (C=O), verbal peak at 1514.24cm^{-1}

Due to absorption of(C=C aromatic),verbal peak at 1616.65 cm^{-1}due to absorption (C=C alkene) , strong peak at 2925.34cm^{-1} due absorption of (C-H alkane stretch) ,weak peak at 2856.89cm^{-1} due to absorption of(C-H aldehayde), strong peak at 1455.17cm^{-1} due to absorption of (C-H alkane), strong peak at 1071.47cm^{-1}due to absorption of (C-O bond) , strong peak at 666.40 due to absorption of (C-H out of plane). And also applied antibacterial test and fungi test give result (Ec, Ps, Sa, Bs and Ca) Give positive result and negative result with tannin at 100mg/ml negative result with Bs but given positive result with Es, Ps, Sa, Ca, at 50mg/ml give positive result with Es, Ps, Sa, Ca but negative result with Bs , at 25mg/ml give positive result with Es, Ps , Sa ,Ca but negative result with Bs, at 12.5mg/ml give positive result with Es, Ps, Ca but negative with Sa, Bs. At 6.25 give positive result with Es, Ps, Ca but given negative result with Sa, Bs, Ca but given negative result with Sa, Bs.

3-5 Conclusions:

The tannin was extracted by separator funnel and the percentage yield of it was fund to be equal 0.426%.

For this extract the IR spectra give wide range of absorption. And also give positive result in anti bacterial and antifungal test.

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