Introduction

1.1 Azadirachtaindica:

1.1.1 Common names:

Neem,neem tree, Indian-lilac (Canada), margosa, nimtree and margosier. In North Queensland, the common name 'neem' is erroneously applied to Meliaazedarach ('white cedar'), a morphologically similar, but smaller, plant.

1.1.2 Classification of Neem Tree

Family: Meliaceae

Sub Family: Melioidear

Tribe: Melieae

Order: Retainer

Genus: Azadirachta

Species:Azadirachtaindica

Common name: Neem , Nim and more than 100 name

1.1.3 Description of Neem tree:

Neem is fast-growing tree, generally 15–20 m tall (sometimes up to 40 m tall), with a crown diameter up to 20 m .Neem is evergreen but can shed most of its leaves under dry conditions. The compound (pinnate) leaves are alternate, 20–40 cm long, with 20–30 dark green, serrated leaflets, each about 3–8 cm long. The terminal leaflet is often absent. Young leaves are reddish to purplish in colour.Petioles are 70–90 mm long. The bark is deeply fissured. Flowers are cream colored; perfumed and arranged in axillary clusters (each cluster is called an inflorescence). Each inflorescence is 15–25 cm long and comprises 150–250 individual flowers. Each flower is about 1 cm in diameter with five petals, ten stamens and one style .The ovary is syncarpous, superior, three-celled with 1–2 ovules per cell. The fruit is a glabrous, olive-like drupe, 1–3 cm in diameter, varying in shape from elongate oval to roundish. It is yellow when ripe and comprises a sweet pulp enclosing a

single seed (rarely 2–3 seeds) .Neem has a strong root system with a deep tap root and extensive lateral roots. Suckers can be produced following damage to the roots (Hearne 1975).

1.1.4 Chemistry of Neem:

Neem elaboratrs a vast of biologically active compounds which are chemically diverse and structurally complex.Neem chemistry dates back to 1880-90 when influenced by its folklore medicinal values, the chemist took up the isolation of active principle from its seed and other parts .Siddiqui was the first to report the isolation of three products viz .nimbin ,nimbidin and nimbinin from its oil (M.Aktar , D Sengupta ,2008).The Neemconstituents belonging to diverse classes have been divided into two major section:

- Isoprenoids and Non- Isoprenoids.

The latter category comprises glycerides, polysaccharides, sulphurones compounds, flavonoids and their glycosides, amino acid, aliphatic compounds est.

1.1.5 Uses of Neem:

Neem trees are grown commercially in plantations to produce azadirachtin, a chemical extracted from the seeds and leaves. Azadirachtin has been promoted as a new insecticide that is considered more 'environmentally friendly' than synthetic insecticides. Plantations have been established in tropical to subtropical regions of the world, including semi-arid and wet tropical regions, from sea level to about 700 m elevation (NRC 1992). After the oil has been pressed from the seeds, the residue ('Neem cake') can be used in cattle and poultry feed. Neem is also used in silviculture in India and for reforestation in Asia, Central America and the sub-Saharan region (Maramoroschn.d.). It has been planted as an ornamental and has been sold bycommercial nurseries in Queensland (Lawson 1997). In some towns and cities, it has been promoted as a street tree (Hearne 1975).Those advocating the planting of Neem as a commercial crop cite a wide range of potential benefits .Some go as far as saying Neem is 'a tree for solving global problems' (NRC 1992). While Neem has a range of potential uses, most commercial interest lies in the pest control properties of Neem extracts. While azadirachtin and other Neem extracts have been

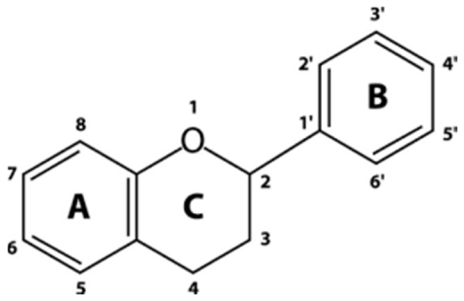
shown to have insecticidal properties, the commercial viability of producing these insecticides appears less certain. In 1988, an economic assessment of Neem concluded that 'Neem has little current demand with no local production and only small volumes of imports.

1.2 Flavonoids:

Are the most abundant polyphenols in human diet, representing about 2/3 of all those ones ingested. Like other phytochemicals, they are the products of secondary metabolism of plants and, currently, it is not possible to determine precisely their number, even if over 4000 have been identified. In fruits and vegetables, they are usually found in the form of glycosides and sometimes as acylglycosides, while acylated, methylated and sulfate molecules are less frequent and in lower concentrations. They are water-soluble and accumulate in cell vacuoles.

1.2.1 Chemical structure of flavonoids:

Fig (1): Skeleton of Diphenylpropane:

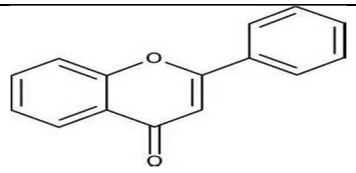
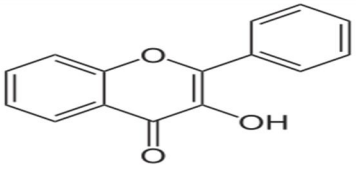
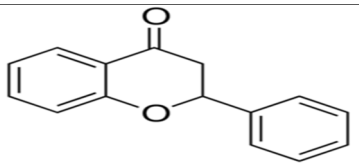
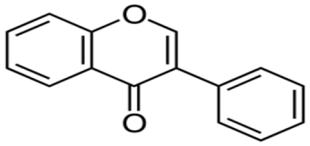
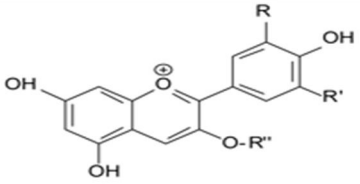
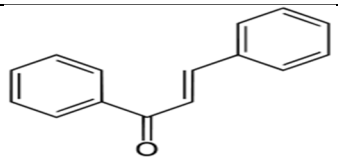
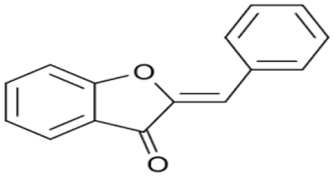


Their basic structure is a skeleton of diphenylpropane, namely, two benzene rings (ring A and B, see figure) linked by a three carbon chain that forms a closed pyran ring (heterocyclic ring containing oxygen, the C ring) with benzoic A ring. Therefore, their structure is also referred to as C6-C3-C6.In most cases, B ring is attached to position 2 of C ring, but it can also bind in position 3 or 4; this, together with the structural features of the ring B and the patterns of glycosylation and hydroxylation of the three rings, makes the

flavonoids one of the larger and more diversified groups of phytochemicals, so not only of polyphenols, in nature. Their biological activities, for example they are potent antioxidants, depend both on the structural characteristics and the pattern of glycosylation (*).

1.2.2 Classification of flavonoids:

Table (2): Basic structure of flavonoid:

Flavonoid	Basic structure of flavonoid
Flavones	
Flavonol	
Flavanone	
Isoflavone	
Anthocyanin	
Chalcone	
Aurane	

Most of them are present in our everyday's life (Manach et al., 2004, Dahan et al., 2004). For instance, flavones, such as luteolin and apigenin glycosides, are contents of parsley and celery. The richest sources of flavonols, like quercetin, are capers, lovage, apples, tea plant, onions, red grapes, citrus fruits, curly kale, leeks, broccoli, cherries, raspberry, cranberry and blueberry. Flavanones are abundant in high concentrations in citrus fruit.

1.2.2.1 Flavones:

They have a double bond between positions 2 and 3 and a ketone in position 4 of the C ring. Most flavones of vegetables and fruits has a hydroxyl group in position 5 of the A ring, while the hydroxylation in other positions, for the most part in position 7 of the A ring or 3, and 4, the B ring may vary according to the taxonomic classification of the particular vegetable or fruit.Glycosylation occurs primarily on position 5 and 7, methylation and acylation on the hydroxyl groups of the B ring. Some flavones, such as nobiletin and tangeretin, are polymethoxylated (*).

1.2.2.2 Flavonols:

Compared to flavones, they have a hydroxyl group in position 3 of the C ring, which may also be glycosylated. Again, like flavones, flavonols are very diverse in methylation and hydroxylation patterns as well, and, considering the different glycosylation patterns, they are perhaps the most common and largest subgroup of flavonoids in fruits and vegetables. For example, quercetin is present in many plant foods (*).

1.2.2.3 Flavanones:

Flavanones, also called dihydroflavones, have the C ring saturated; therefore, unlike flavones, the double bond between positions 2 and 3 is saturated and this is the only structural difference between the two subgroups of flavonoids. The flavanones can be multi-hydroxylated, and several hydroxyl groups can be glycosylated and/or methylated. Some have unique patterns of substitution, for example, furanoflavanones , prenylatedflavanones, pyranoflavanones or benzylatedflavanones, giving a great number of substituted derivatives. Over the past 15 years, the number of flavanones discovered is significantly increased (*).

1.2.2.4 Flavanonols:

Flavanonols, also called dihydroflavonols, are the 3-hydroxy derivatives of flavanones; they are an highly diversified and multisubstituted subgroup (*).

1.2.2.5 Isoflavones:

As anticipated, isoflavones are a subgroup of flavonoids in which the B ring is attached to position 3 of the C ring. They have structural similarities to estrogens, such as estradiol, and for this reason they are also called phytoestrogens (*).

1.2.2.6 Neoflavonoids

They have the B ring attached to position 4 of the C ring (*).

1.2.2.7 Anthocyanidins:

Chemically, anthocyanidins are flavyliumcations and are generally present as chloride salts. They are the only group of flavonoids that gives plants colors (all other flavonoids are colorless). Anthocyanins are glycosides of anthocyanidins. Sugar units are bound mostly to position 3 of the C ring and they are often conjugated with phenolic acids, such as ferulicacid.The color of the anthocyanins depends on the pH and also by methylation or acylation at the hydroxyl groups on the A and B rings (*).

1.2.2.8 Chalcones:

Chalcones and dihydrochalcones are flavonoids with open structure; they are classified as flavonoids because they have similar synthetic pathways (*).

1.2.3 Medicinal properties of flavonoids:

Oxidative stress is considered to be substantial, if not crucial, in initiating and developing of many conditions and diseases of modern time: inflammation, autoimmune diseases, cataract, cancer, Parkinson's disease arteriosclerosis and aging. Having all that in mind, it is reasonable to believe that exogenous antioxidants could play important role in preventing oxidative damage in cells and tissues. Flavonoids are well known for their antioxidant activity. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Kukić et al., 2006).Epidemiological studies have described the beneficial effects of dietary

polyphenols(flavonoids) on reduction of chronic diseases, including cancer (Ramos, 2007).Luteolin is a flavone with important role as antioxidant, free radical scavenger, anti-inflammatermagant, immune system modulator and cancer prevention agent. Flavonolquercetin it the most active of the flavonoids. Many medicinal plants owe their activity to their high quercetin content. Several studies revealed quercetin's significant anti-inflammatory activity due to direct inhibition of initial processes of inflammation. Potent antioxidant activity of quercetin is demonstrated, too. Some tests showed antitumor properties oquercetin including the inhibition of cancer cell proliferation and migration (Lim et al., 2006).Problem with this new type of chemotherapeutic agent is low potency and poor selectivity. Combinatorial application of quercetinand ultrasound on skin and prostate cancer showed 90% mortality within 48 hours with no visible mortality on normal cells (Paliwal et al., 2005). In addition, quercetin may have positive effects onprostatis, heart disease, cataracts, allergies, bronchitis and asthma. Genistein and daidzein, well known soyaisoflavones, function as antioxidants, antiestrogen, antiangiogenic and anticancer agents. For later, it is reported that genistein exhibits significant reduction (44%) in experimental lung tumor metastasis (Ogasawara et al., 2007).Flavan-3-ols from tea, cocoa, chocolate, fruits, vegetables and wine, are highly potent antioxidant compounds. They reduce incidence of stroke, heartfailure and diabetes and cancer. Their anticancer effects are thoroughly investigated. Epigallocatechin 3-gallate and gallocatechan 3-gallate induces reduction in experimental lungtumor metastasis (77% and 46%). Epigallocatechin3-gallate is effective ant angiogenesis agent which inhibits tumor cell invasion and proliferation (Ogasawara et al., 2007 & Tang et al., 2006). It ,also, inhibits growth of the NBT-II bladder tumor cells and breast cancer cell lines (Chen et al., 2004& Kavanagh et al., 2001). Cyanidin is anthocyanin pigment found in many berries (grapes, blackberry, blueberry, cherry, cranberry, raspberry etc.), apples, plums and redcabbage. It exerts antioxidant, anti-inflammatory and anticancer effects. It may have an important role in future cancer treatment (Fimognari et al, 2005).

Material and method

2.1 Material:

2.1.1 Chemical:

Silica gel, distal water, Ethanol, Butanol, Acidic acid.

2.1.2 Instrumental use:

Thin layer chromatography and Infrared technique.

2.2 Method:

2.2.1 Collection of the plant material:

Fresh leaves of Neem (azadirachtaindica) which are green in color were collected from Khartoum north city (Sudan), the leaves were washed with water to removed dust ,practical Healthy green leaves were used in this study .the leaves were dried in air and shade at room temperature for 3 days ,after drying the crushes leaves were powdered .

2.2.2 Preparation of Neem of extracts:

300g of the powdered sample was weighed. The weighed sample was macered in ethanol (1 litter) for 48 hours with shake. After that, the sample was Filtered and coped to Petri-dishes to evaporate ethanol then crude was obtained.

2.2.3 The extraction of flavonoids from Neem:

2.2.3.1 Thin-layer chromatography:

Silica gel and water (1:2; W: V) were mixed to from slurry this was separated over clean glass plates. These plates were employed for chromatographic fractionation without activation. (0.2g)of the crude product was dissolved in the minimum amount of 95% ethanol and applied as concentrated spot on the **TLC** plates .the plates were developed

with different solvent systems in rat of butanol : acetic acid :water (6:2:4 ;v:v:v), (5: 1:4 ;v:v:v), (4: 1:6 ;v:v:v), and (5:1:6 ;v:v:v),on Silica Gel Plate As Narrow strips. The plates were developed, after evaporated of the solvent the pure compound was obtained, however the system achieved the best separation solvent was butanol : acetic acid :water (5: 1:6 ;v:v:v).only on major spot was observed when the plates were viewed under IR spectral.

2.2.4 In vitro Antimicrobial activity :

Antimicrobial activity is measured *in vitro* in order to determine:

a. The potency of an antimicrobial agent in solution. Sensitivity tests are also used to evaluate new antimicrobial agents by testing them against large number of organisms of known susceptibility and to compare these results with drugs already available.

b. Its concentration in body fluids or tissue.

c. The sensitivity of a given microorganism to known concentration of the drug.

2.2.4.1 Dilution methods:

The aim of broth and agar dilution methods is to determine the lowest concentration of the assayed antimicrobial agent (minimal inhibitory concentration, MIC) that, under defined test conditions, inhibits the visible growth of the bacterium being investigated. MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents.

In the agar dilution method, the medium is inoculated with the test organism and the samples to be tested are mixed with the inoculated medium. The material is inoculated and the growth of the microorganism is viewed and compared with control culture which does not contain the tested sample. The experiment is repeated at various dilution of the test sample in the culture medium and the highest dilution at which the sample just prevents the growth of the microorganism (MIC) is determined.

Dilution tests on solid media involve addition of varying concentrations of drug to measured volumes of agar medium which has been melted and cooled to 45-50° C, the resultant mixtures are then poured as plates into Petri-dishes or as slants into test tubes. Standardized inocula are seeded onto the surface of the medium and MIC read after an appropriate incubation period In these methods, it is essential to test strains of known susceptibility with each series of unknowns in order to be sure against drug deterioration, in- accuracies in dilution or variation in the medium.

Breakpoint concentrations of antibiotics are used to characterize antibiotic activity: the interpretive categories are susceptible, moderately susceptible (intermediate), and resistant. These concentrations are determined by considering pharmacokinetics, serum and tissue concentrations following normal doses, and the population distribution of MICs of a group of bacteria for a given drug.

2.2.4.2 Determination of minimum inhibitory concentrations (MICs) by agar plate dilution method:

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 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.

2.2.5 Testing of antibacterial susceptibility

2.2.5.1 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 108cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial (Bacillus subtilis , Staphylococcus aureus and Escherichia coli , Pseudomonas aeruginosa) 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.

2.2.6 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 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108- 109 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.

2.2.7 Preparation of fungal suspension:

The fungal cultures (Candida albicans) 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.

Result and discussion

3.1 antibacterial and antifungal activities:

Using dis diffusion method to screen the antibacterial and antifungal acting of plant extracts , the test result are depicted in table with different concentration of Neem extract are tested against negative bacteria Escherichia col (Ec) and Pseudomonas aeruginosa (Ps) mgram postive bacterial Staphylococcus aureus (Sa) and Bacillus subtilis (Bs), gram negative bacterial and anti-fungi Candida albicans (Ca) . The testy results revealed that the high concentrated of Neem extract showed moderate activity as anit the tow bacterial species and fungi.

At 100mg/ml concentration of neem extract was found to be the antibacterial activity in Ec, Ps, Sa and Ca was active, and the antibacterial activity in BS was very active.

From These results can be used Neem leaves in medical treatment.

Table (2): antibacterial activity of azadirachtaindica against stander organism

Neen crude	**Mean diameters of inhibitory zone (mm)**				
	Ec	**Ps**	**Sa**	**Bs**	**Ca**
100 mg\ml	18	18	15	23	18
50 mg\ml	16	17	14	21	17
25 mg\ml	14	16	13	14	16
12.5 mg\ml	12	15	11	12	15
6.25 mg\ml	10	14	10	11	14

(Ec) =Escherichia col

(Ps) =Pseudomonas aeruginosa

(Sa)=Staphylococcus aureus

(Bs) = Bacillus subtilis

(Ca) = Candida albicans

The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active. (Sana Mukhtar ,Ifra Ghori (2012).

3.2 Spectral Dataof flavonoid:

From the IR spectrum there found the broad intense beak in arrange between 3500 – 3000 is show of there is O-H group .and the beak at as 2924.02 is indicate the C-H group,and the beak at as 1726.32, is indicate the C=O stretch ,and the beak at as 1600 is indicate the aromatic C=C bending group,

The IR spectrum of flavonoid (fig-2)

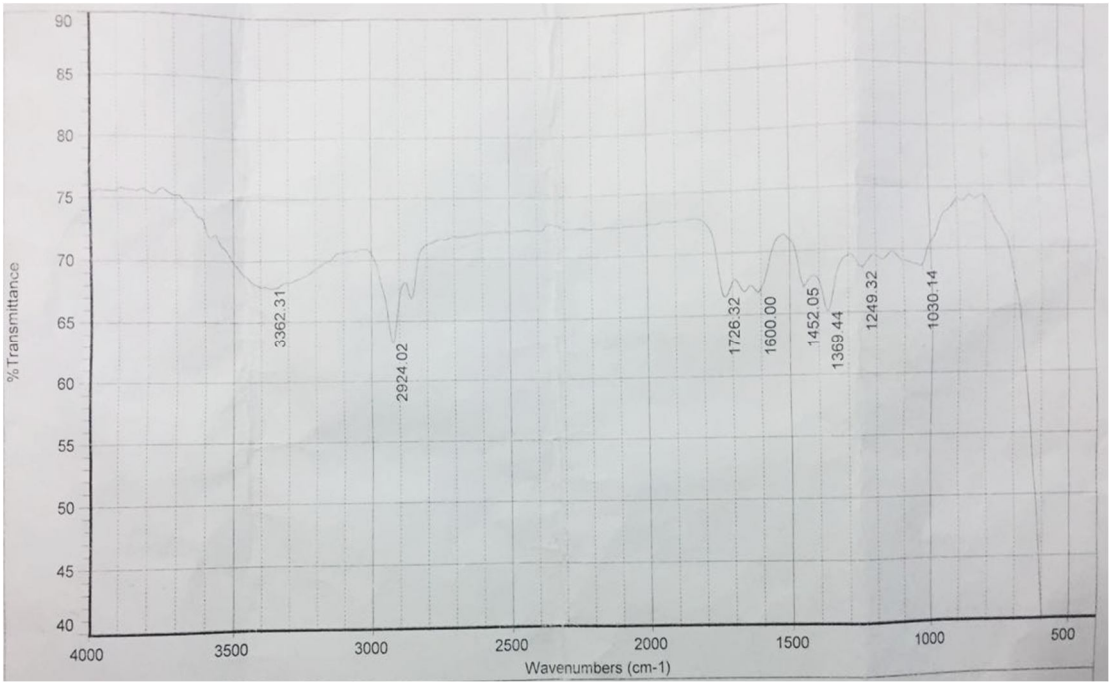


Fig: (2) Inhibition zone of crude and pure compound of the leaves of Neem against Candida albicans

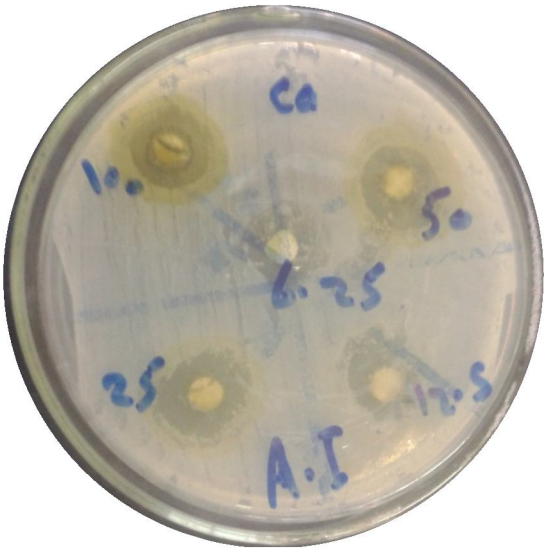


Fig: (3)Inhibition zone of crude and pure compound of the leaves of Neem againstEscherichia coli

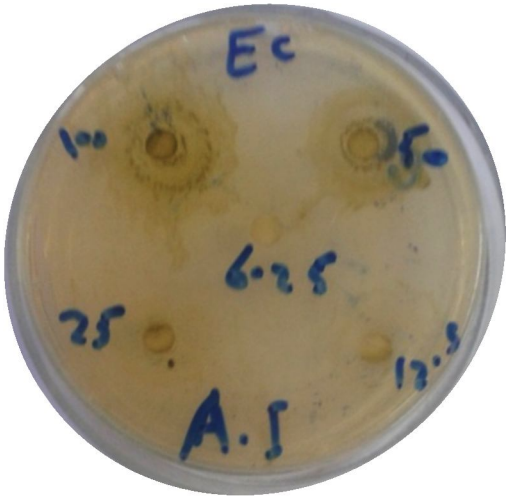


Fig: (4) Inhibition zone of crude and pure compound of the leaves of Neem against Pseudomonas aeruginosa

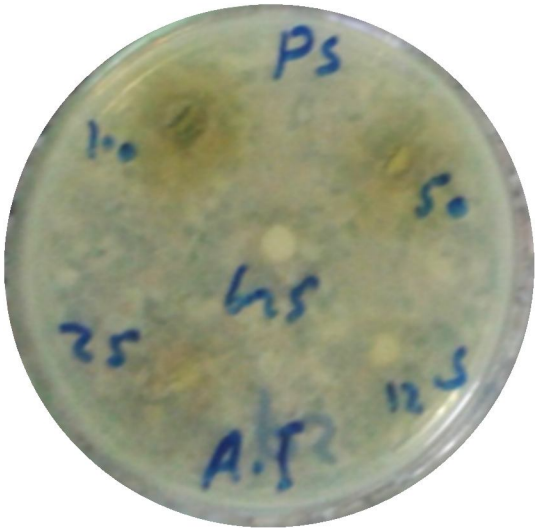
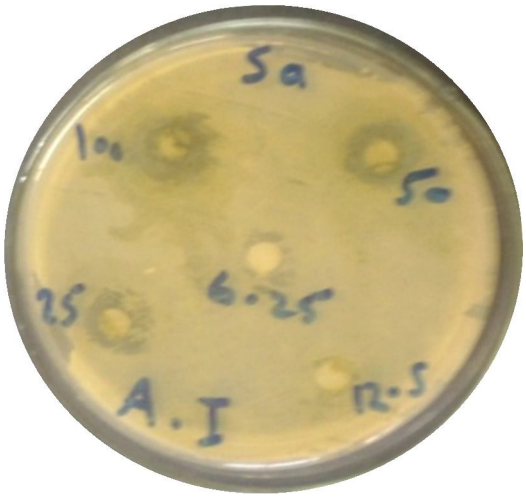


Fig: (5)Inhibition zone of crude and pure compound of the leaves of Neem against Bacillus subtilis

Fig: (6)Inhibition zone of crude and pure compound of the leaves of Neem againstStaphylococcus aureus



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