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ANTIMICROBIAL ACTIVITY OF COLD PRESSING OIL OF NIGELLA SATIVA, PAPAVER SOMNIFERUM, PRUNUS AMYGDALUS, SESAMUM INDICUM

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ABSTRACT

This study is investigate anti-microbial activity and GC-MS analysis of cold-pressed oil that extracted from each of Nigella sativa (Çörekotu), Prunus amygdales (Acı badem), Sesame (Susam) and Papaver somniferum (Haşhaş).

We tested against: Gram-positive bacteria: Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus Faecium, Enterococcus Faecalis, Klebsella pneumoniae, bacillus subtilis. Gram negative bacteria: Salmonella typhimurium, Salmonella Kentucky, Salmonella infantis, Salmonella enterocolitis, Enterobacter aerogenes, Pseudomonas aeruginosa, Pseudomonas fluorescens and fungi: Candida albicans.

Keywords: Nigella sativa, Sesame, Prunus amygdalus, Papaver somniferum, Antimicrobial, GC-MS.

* INTRODUCTION

The human body is made up of several systems that all plays together as a unit to make sure that the body functioning well. And each organ has its own specific function. Thus, all precautions required should be taken to protect them from various risks, including malnutrition, which results in a weakened immune system of the body.

Whereas, the immune system is the first line of defense for the human body against any attack from the causes of ill-health, which invade the human body, for example, but not limited to, pathogenic bacteria,

viruses, or other microorganisms that can cause disease.

There are numerous of ways to strengthen the immune system, including the use of available vaccinations of modern medicine for some disease. In addition to, some medicinal and aromatic plants, which is one of the traditional crops, It has been used by mankind over the centuries in various purposes. Sometimes used as a spice when cooking foods, a kind of medicine in medieval, nowadays, was clearly evident how important is the medicinal and aromatic plants in the treatment of many human diseases.

Medical plant is everything considered as plant origin and used medically in a treating a particular disease or reduce injury, and the raw materials which they contains are used in the preparation of medical materials. In addition to their capability of treating a particular disease or reduce injury.

The aromatic plant is any plant contains the aromatic oil "volatile oil" in part that uses in the preparation of the perfume and there are plants containing essential oil. Considered medicinal and aromatic plants of the most important strategic materials in the pharmaceutical industry, the factors that led to increased interest in the cultivation of medicinal and aromatic plants use in the treatment of diseases in the last period following:

First, experience has shown that the influence of the material of different effective in the laboratory does not lead to physiological influence played by the same active ingredient derived from medicinal plants note that Article different lab is a high degree of purity.

Secondly, experience has shown that compounds that prepared in the lab have many side effects basic medical impact that is used for in most cases, these effects are harmful. These are known reasons for that God Almighty had created in a single plant the contents of the full prescription of more than one active ingredient that these substances work together collaborating in the treatment of disease and obtain some in the pure suit, its use alone is the one that does not lead effectiveness or effects harmful side.

*** Preface Nigella Sativa**

Our prophet Mohammed said: (Black cumin cures all malady except the death) [Al-Bukhari and Muslim] [1]. For this reason, I have a curiosity to study this plant.

A lot of medicinal plants and their pure ingredients have been shown beneficial curative potentials. Seeds of Nigella sativa, a dicotyledon

of the Ranunculaceae family, have been utilised for thousands of years as a flavouring and food preservative. The oil and seed ingredients, in particular, thymoquinone (TQ) (Figure 1.1) [2], have shown medicinal characterises, in traditional medicine [3]. As we noticed in the recent history there are a lot of findings provide clear evidence that both the oil and its active ingredients Importance, especially, thymoquinone, own reproducible anti-oxidant effects through augment the oxidant scavenger system, which as a consequence lead to antitoxic effects [2, 4]. The oil and thymoquinone of Nigella Sativa (Çörekotu Tohumu**)** content have effective anti-inflammatory effects on numerous inflammation-based including experimental of Encephalomyelitis, Colitis, Peritonitis,inhibition of Oedema and Arthritis into abolition of the inflammatory prostaglandins and leukotrien. The oil and active elements have given good properties for immunity system by raise the T cell and natural killer cell mediated immune responses and not only that the most important, in Nigella sativa both the oil and its active elements represent antimicrobial and anti-tumor characteristic against various microbes and cancers [2, 5].

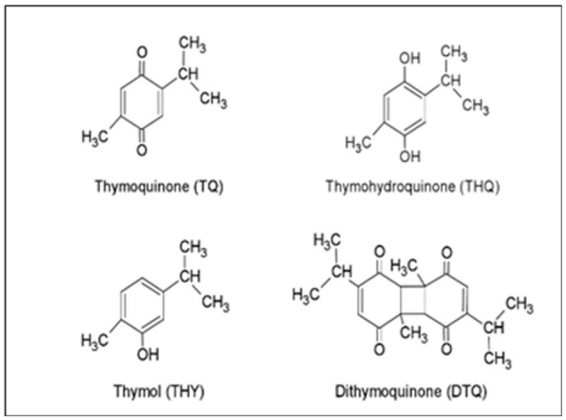


Figure 1.1. Chemical structure of Thymoquinone (TQ).

Black cumin, (Nigella sativa) is widely grown in different parts of the world and the seed of black cumin (Nigella sativa) has been used to enhance health for countries in particular, in the Middle East and Southeast Asia [6]. Black cumin seeds have been widely used in traditional medicine as diuretic and antihypertensive [7], digestive and appetite stimulant [8], antidiarrheal [9],analgesic [5], anthelmintic [10] and antibacterial. Additionally, recent studies have shown black cumin to be antidiabetic [11], anticancer, anti-inflammatory, spasmolytic and bronchodilatory [9, 12], hepatoprotective [13, 14]. Renal protective [15] and possessing antioxidant properties [7]. Black cumin(Nigella sativa) its seeds are consist of a Volatile oil (0,5; 1,6%), a Fixed oil (35,6-41,6%), Protein (22,7%) [14] and Amino acids [14, 16]. In addition, the seeds of black cumin (N.sativa) consist fat, crude

fibre, minerals; for instance Na, Cu, Zn, Ca, Fe, P and vitamins (Thiamine, niacin, pyridoxine, Ascorbic acid and Folic acid) [14, 17], Black cumin (Nigella sativa seeds) output esters of fatty acids, free sterols and sterol esters [14, 18].

*** Preface Sesame (Sesamum Indicum L)**

Sesamum is most common in equatorial regions everywhere in the world since old centuries. The Sesame seeds are prosperous exporter of protein. İn addition, it is one of the most product provide for oil production [19]. Sesame includes the essential fatty acids (EFAs) [19] e.g. linoleic acid and high levels of lignans that contain of sesamin, sesamol and sesamolin. Sesame adds its use in food also is a part in a soap, cosmetics, lubricants and medicines. The Sesame seeds moreover consist two rare substances are sesamin and sesamolin have a good effect on cholesterol-lowering in humans and to limit high blood pressure disease [19].

Sesame [20] seed has oil content (around 50%) than most of the known oil-seeds although its production is far less than the major oil seeds such as soybean or rapeseed, sesame oil is generally noted as high-cost and high-quality oil, it is one of the most stable edible oil despite its high degree of unsaturation, a presence of type natural antioxidants it is lignan of sesame oil and has useful physiological effects. In addition, these were note reported to increase the hepatic mitochondrial and the peroxisomal fatty acid oxidation rate in laboratory animals [19, 21]. Cephalin, a phospholipid from sesame seed has been reported to possess hemostatic activity. The oil also known as gingelly oil or til oil. In addition, the sesame has large-scale medical and pharmaceutical applications. The least that can be said it is laxative, emollient and demulcent. Sesame seed and its green leaves may be used as a medicineis reflected the antibacterial activity of Sesame seeds against Staphylococcus and Streptococcus as well as common skin fungi, such as athlete's foot fungus has also been well recognized [22]. The Sesame oil is used to control in both the High-Density Lipoprotein cholesterol (HDL) and the lower Low-Density Lipoprotein cholesterol (LDL). Pure sesame oil is prosperous with antioxidant ingredients like lignans allowing for greater shelf-life of foods. Additionally improving their flavour and savour, The sesame use as antioxidant also the sesame oil includes a large quantity of linoleate in triglyceride that selectively inhibits

malignant melanoma growth. Off - late, the work has also been oriented towards the production of biodiesel from sesame seed oil as a viable alternative to the diesel fuel [23]. The ethnobotanical and medicinal uses of this commercially necessary, nutritionally rich oil seed need to be investigated for preferable employment [19, 21].

*** Preface Almond (Prunus amygdalus)**

Prunus amygdalus is referred to the Rosaceae family [24]. Almond core contains high level of unsaturated fatty acids, mainly mono-unsaturated fatty acids (MUFA) that perform a vital role in body diet, food [25].

Almond is an exporter of food and medicine, they extend from India to Persia, the tree had spread to east and occident of its region thousands of years. Almond is good sources of antioxidant nutrients. Almonds (Prunus amygdalus) involvement proteins, fiber, vitamin and certain minerals e.g. magnesium and calcium, potassium, low in saturated fatty acids and rich in unsaturated fatty acids [26, 27], for this reason it is reduce coronary heart disease risk factors [28].

Almonds (Prunus amygdalus) are a useful food a cure for anaemia. It's beneficial in the remediation of constipation and various skin diseases like eczema, pimples. Almonds are helpful in heal gastroenteritis, kidney pains, diabetes, head lice, facial neuralgia and gastric ulcer and wound healing, skin cleaner, chapped lips and hand [27, 29].

Oil of Almond had used for the skin as a moisturiser which curbs the skin from drying and peeling skin, from old, Prunus amygdalus oil had used as the comforting cure for skin allergies, and to treat minor hurt. In addition, most widespread use of Prunus amygdalus oil is in massage because it is outstanding skin lotion [29]. Its properties make it popular with massage therapists' worldwide. Almond oil of seed does not have any oleaginous effect and will take a tiny bit of time before it is absorbed by the skin. utilise it for a massage makes a human body feel comfortable [29, 30].

*** Preface Opium Poppy (Papaver Somniferum L)**

It is famous delicacy food, used for direct consumption. Poppy plants have the ability to collect heavy metals [31]. Papaver somniferum one of the most medical seeds useful variety [32]. Opium poppy belong to the sction papaver of the tribe papavereae, and of sub-family papaveroideae [33].

Papaver somniferum (poppy) is most common crop in countries such as China, India, Czechoslovakia or Turkey. Poppy is grown mainly for its content of opium and oil seeds. In addition the seeds of Poppy are used approximately particularly for their oil [34]. The seeds of Poppy include to 50% of oil and have high levels of oleic and linoleic acids, aforementioned crop as a source of linoleic acid, this crop is distributed among the important [35] industrial oil plants in Turkey. Poppy seed oil appears to be of good quality for human consumption since it is generally rich in polyunsaturated fatty acids [36, 37].

* MATERIAL AND METHODS

* Plant Material

The plants used as seeds: Nigella sativa, Sesame, Papaver somniferum, Prunus Amygdalus without crushed (Table 2.1)(Photo 2.1).

Table 2.1. Medicinal plants tested for antibacterial activity using disc diffusion method.

Latin Plant Name	Turkish Plant Name	Plant Part	Metodology	Antimicrobial activity		
				Antibacterial		fungi
				Gram-positive	Gram nagtive	
Amygdalus amara. E1	Acı bademtohumu	Seed	Cold Press	*Staph y. aureus, s. epider. E.Faeciu, E,faecalis, Klebsiella pneumoni, bacillus subtilis.*	*S. typh, S. Kentucky, Salmonella infantis, Salmonella enterocolitis, E. coli, E. aerogenes, P. aeruginosa, p. Fluorescens.*	*C. A*
.Nigella sativa. E2	Çörek otu Tohumu	Seed	Cold press			
Sesamum. E3	Susam Tohumu	Seed	Cold Press			
Papaver somniferum black. E4 Papavar sonmiferum white. E5	Haşhaş Tohumu	Seed	Cold Press			

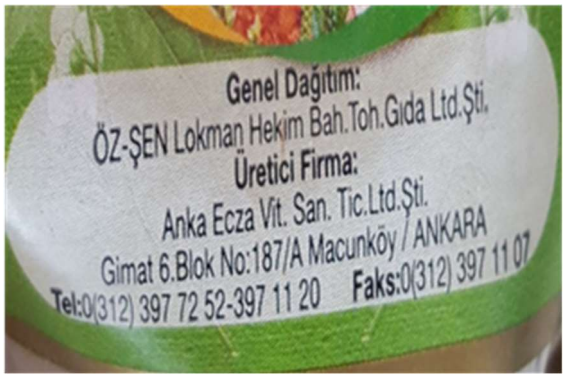


Photo 2.1. Seeds company.

* Preparation of Crude Extracts

Extract cold press oil from seeds: Cold pressing by used pressing machine oil

(Photo 2.2) to separate oil of: -

Photo 2.2. Pressing machine oil.

Using 1030.5g of the Nigella sativa generated about 17% oil and about 83% were dry garage (Photo 2.3).

Photo 2.3. Nigella sativa oil.

Three kg of Sesame gave oil 80% and dry garbage about 20% (Photo 2.4).

Photo 2.4. Sesame oil.

Three kg of Papaver somniferum gave 50% garbage, 50% oil (Photo 2.5).

Photo 2.5. Papaver Somniferum White and Black Oil and Garbag.

Two kg of Prunus Amygdalus gave 60% oil and 40% garbage (Photo 2.6).

Photo 2.6. Prunus Amygdalus oil And Garbage.

*** Prepare oil extracted on the discs**

We put the oil that extracted on filtration papers its diameters are 6mm. Prepared about 50 papers in sterile Petri dishes from each extract with different concentration 15 µg/disc and 5 µg/disc then kept at room temperature ın sterile cabin (Photo 2.7).

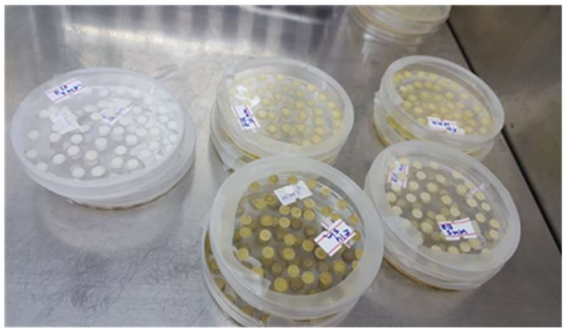


Photo 2.7. Prepare The Extract On Filter Paper.

*** Microbial Material**

Strains of fungi and bacteria obtained from the biology Laboratory in elmergib University: -

1- Srains Gram-positive bacteria: Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis DSMZ 20044, Enterococcus faecium, Enterococcus faecalis ATCC 29212, Bacillus subtilisA DSMZ 1971 (Table 2.2).

2- Strains Gram-negative bacteria: Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, Salmonella enterlidis SL 1344, E.coli ATCC 25922 [96], Enterobacter aerogenes ATCC 13048, Pseudomonas aeruginosa, Pseudomonas fluorescens DSMZ 50071, Klebsiella pneumonia ATCC 7544 (Table 2.3).

And fungi: Candida albicans DSMZ 1386.

Table 2.2. Classification of Gram Positive Bacteria.

Gram Positive Bacteria [100, 102]			
Name	**Morphology**	**Sites of-Transmission**	**Type of Infection**
Staphylococci [97].	*Cocci* in grapelike clusters	Skin,nares/ endogenous, frontal connect, atmosphere air	Soft tissue, bone, joint, endocarditis, food poisoning [98]
Enterococci	Cocci in pairs, chains	GI tract, endogenous, direct contact	UTI, GI, catheterrelated infections
Bacilli	Rods, sporeforming	Soil, air, water, animals / aerosol, contact	Anthrax, food poisoning, catheter-related infections [97].

Table 2.3. Classification of Gram Negative Bacteria.

Gram Negative Bacteria[97]			
Name	**Morphology**	**Sites of- Transmission**	**Type of Infection**
Enterobacteriaceae (E. coli, klebsiella, salmonella, shigella)	Rods	GI tract, animals / Endogenous, fecaloral [97].	Diarrhea, urinary tract, food poisoning, sepsis [97].
Pseudomonas [97]	Rods	Water, soil / Endogenous, breach of skin barrier [97].	Infections in immunocompro mised hosts, Cystic Fibrosis [97].

* Prepare of microorganisms

A bacteria culture (which has been adjusted to 0.5 McFarland standards (1.0 x 108 CFU/ml) measured using the Turbidimeter (Oxoid, UK). Took swab of the bacterial and fungal suspensions by sterile swab, then mixed with 250ml of distilled water and 2ml of NACL (saline solution) in the test tube (Photo 2.8). After that, wrote the bacteria and fungi names on the tubes, then mixed by the shaker before used.

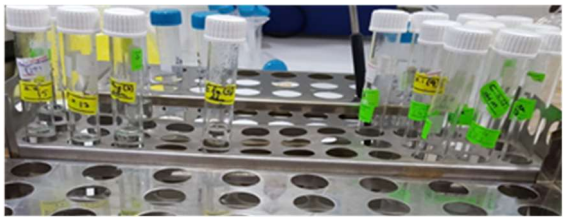


Photo 2.8. Prepare microorganisms in test tube.

* Antimicrobial Activity of Extracts Oil

Petri dishes with Mueller-Hinton agar bought ready (Photo 2..9), Test tube of bacterial and fungi, sterile swabs, Forceps cellulose discs 6mm contain oil that extracted from seeds, Benchtop sanitizer, and Alcohol burner.

* Disc Diffusion Method (DDM)

Diffusion method adopted according to Kavanagh. We have prepared a petri dish, then took anointed of the bacteria and fungi that prepared in a test tube by sterile swabs. Wipe swap on plate gently by spreading process, rotate the plate 60 degrees clockwise and again spread the bacteria going left to right, top to bottom (Photo 2.10) After that, distributed the discs that contain extracts (oil) by a sterile needle with different concentration 15 µg/disc, 5 µg/disc and zero for control, plates were incubated at 37°C for 18 to 24 (Photo 2.11). After the incubation, the plates were examined for inhibition zone. The inhibition zone was measured by using a ruler and recorded. The test was repeated three

times to ensure reliability (Photo 2.12).

Photo 2.9. Mueller-Hinton agars.

Photo 2.10. Swap plate gently.

Photo 2.11. The discs and how kept in an incubator.

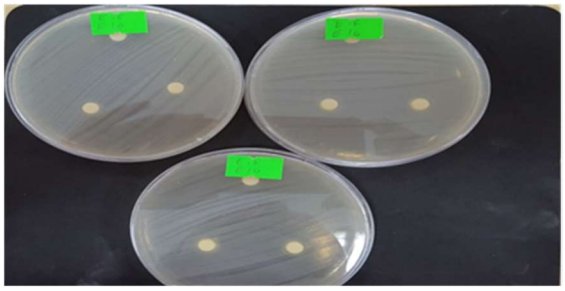


Photo 2.12. Repeated three times.

*** Minimum Inhibition Concentrations (MIC's)**

The minimal inhibitory concentration (MIC) of an antimicrobial agent is the lowest (i.e. minimal). We determine the concentration in the laboratory by incubating a known quantity of bacteria with specified dilutions of the antimicrobial agent. this method has just used with samples have a doubt with their results [99, 100]. Have to follow the instructions to get results valid and reliable of any susceptibility test for detecting antimicrobial-resistant bacteria.

The extracts added into the Mueller-Hinton agar that prepared of a dehydrated base, the pH of the agar must be between 7.2 and 7.4 at room temperature which each plate containing a different concentration of the extract (Photo 3.13). Within 15 minutes of adjusting the inoculum to the 0.5McFarland turbidity standards, mixed the suspension and dilute it so that the final concentration in each well is 5 ×10 CFU/ml. Deliver 2.0 mL of the original suspension into 38 mL of water (1:20 dilution) [100, 101].

The inoculator of the prongs will transfer 0.01 mL (1:10 dilution) into each well. Inoculate MIC panel carefully to avoid splashing from one well to another (Fig 3.1) [48, 100]. After incubated 18-24 hours at 37˚C temperature, checked the MIC endpoint as the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism as detected by the unaided eye.

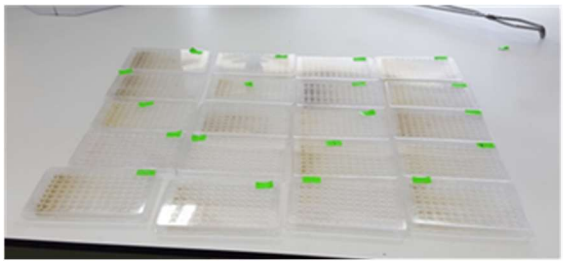


Photo 2.13. MIC.

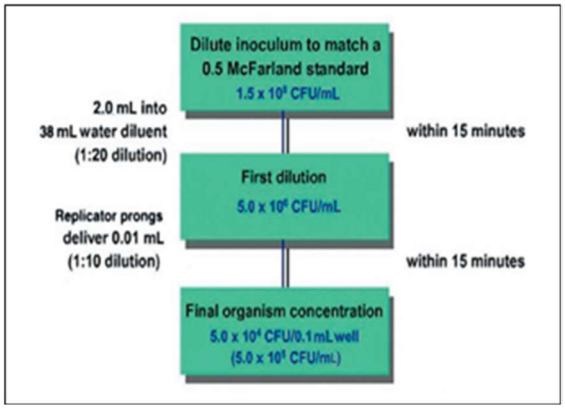


Figure 2.1. Dilution scheme for preparing a standardized inoculum for MIC tests [100].

*** GC-MS Analysis**

For the identification of chemical components, each sample was analyzed by GCMS QP 2010 Ultra (Shimadzu) equipped with Rtx-5MS capillary column (30m·0.25 mm; coating thickness 0.25 μm). Analytical conditions were injector temperature, 250 ∘C; carrier gas Helium at 1 mL/min; injection mode: split, split ratio 1:10; volume injected: 1 μL of a solution in hexane of the oil; and oven temperature programmed from 40∘C to 240˚C at 4∘C/min, pressure:100kPa, purge flow:3 ml/min. The MS scan conditions used included a transfer line temperature of 250˚C, an interface temperature of 250˚C, an ion source temperature of 200˚C. Identification of the constituents was based on comparison of the retention times and on computer matching against Wiley Data library. When possible reference compounds were cochromatographed to confirm GC retention times.

*** RESULTS**

*** Antimicrobial Activity Test**

In this research, the purpose to examine the inhibitory effects by using an oil that extracted of some medicinal plants in a way that cold pressure using a cold pressing machine of Sesamum indicum, Nigella sative, Prunus amygdalus, white and black Papaver somniferum were tested against fifteen microorganisms (Srains Gram-positive, Gram-negative and Candida albicanis). By Disc Diffusion Method have noted in Black Papaver somniferum (poppy) had affected against fifteen microorganisms, average of inhibition zone in 5 concentration was between 2-8mm and 15 concentration was between 7-9 as shown in (Table 3.1) and (Photo 3.1). A level of Significance was 0.00, according to Spss analysis as shown in (Table 3.2) ,the highest antimicrobials activity was against E.aerogens, K. pneumonia 9.0±1.0 in 15 con higer then 5 con 7.5±0.5 while the lowest one was on C. albicanis,

E. faecalis as shown in (Table 3.3) wheares was in 5 con 2,033±3,52184, 2.0667±3,57957, respectively.

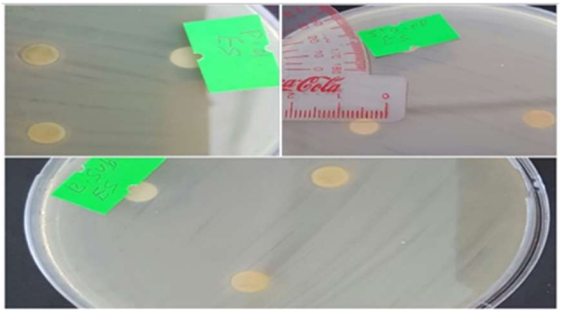


Photo 3.1. Black Papaver Inhibition Zone.

Table 3.1. Antimicrobial activity of the oil extracted seeds by Disc diffusion method.

Mean Diameter of Growth Inhibition Zone (mm)				
Microbial	*Prunus amygdalus* 5 15 µg/disc	*Nigella sativa* 5 15 µg/disc	*Sesame & white papaver somniferum* 5 15 µg/disc	*Black Papaver somniferum* 5 15 µg/disc
S. enteritis	7.1 7.5	- 2	-	7.5 8.5
C. albicanis	-	- -	-	2 7.1
Staph. aureus	-	- -	-	7.1 7.5
E. faecium	7.5 8.5	- -	- -	7.5 8.5
E. faecalis	7.1 7.5	- -	- -	2 8.5
S. typhimirium	7.1 7.5	- -	- -	7.5 8.5
E.aerogens	7.1 7.5	- 6.8	- -	7.5 9
S. infantis	7.1 7.5	- 6.8	- -	7.5 8.5
S. Kentucky	6.7 7.5	- -	- -	7.5 8.5
Pseud.floverscans	- 8.5	- -	- -	6.8 8.5
Kleb.pneumonia	6.7 7.5	- -	- -	7.5 9
B. subtilis	-	- -	- -	7.5 8.5
Staph.epidermis	-	- -	- -	6.7 8.5
Pseudomonas arginosa				7.5 8.5
E.coli	-	- -	- -	6.7 8.5

(-): No affect.

*** statistical Analysis**

ANOVA, Decriptive and Homogeneous were performed to test for defferencd in size of inhibitory zone formed by oil for Black Papaver somniferum and Prunus amygdlus against ddifferent bacteria by IBM spss version 24.

Antibacterial activities of P. Amygdalus samples against some of microbial no inhibition zone with B. subtilis DSMZ 1971, S.epidermi DSMZ 20044, P. aerginosa DSMZ 50071, E. col ATCC 25922i, S. aureus ATCC 25923 and C. albicanis DSMZ 1386. Antibacterial activities of P. amygdalus was lower than P. somniferum the average of inhibition zone ranging (7-8mm) as (Photo 3.2-3.3) and (Graphics 3.1-3.11). A level of Significance was 0.00, according to Spss analysis as shown in (Table 3.3) while was the highest antimicrobial activity against P. flore in 15µ/disc cons about 8,5±0,4 and on E. facium about 8,5±0,5 as shown in (Table 3.5).

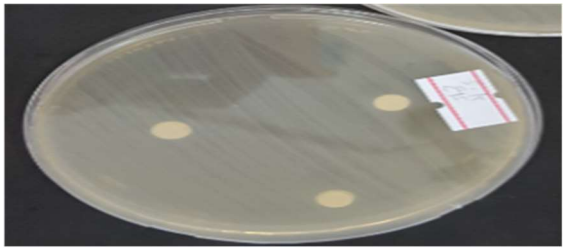


Photo 3.2. Antibacterial Activities of P. Amygdalus.

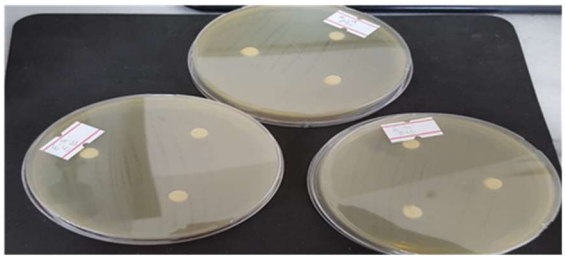


Photo 3.3. Inhibition Zone of P. Amygdalus.

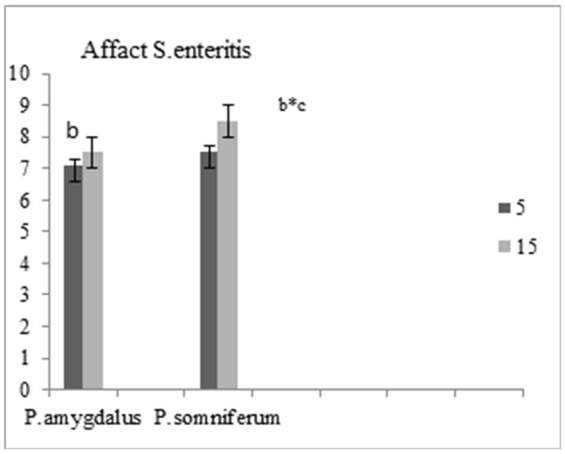
Table 3.4. Prunus amyglus inhibition zone (ANOVA).

	Sum of Squares	Degree freedom	Mean Square	F valu	Level of Significance
Between Groups	167,588	17	9,858	63,830	,000
Within Groups	5,560	36	,154		
Total	173,148	53			

Table 4.5. Papaver inhibiton zone (Duncan test) and std. Deviation.

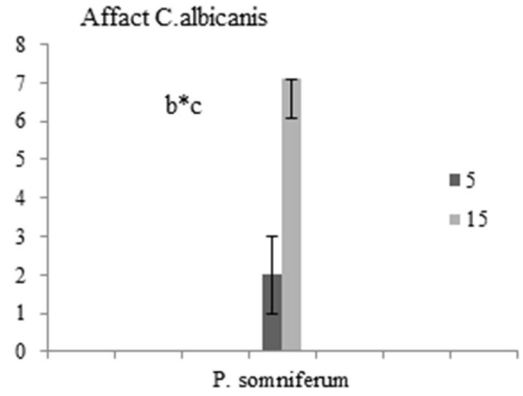
Microornanis	µ/ml	N	Subset for alpha = 0.05			mean±Sd. Deviation
			a	b	c	
Pseud.Flov	5	3	,0000			,0000±0,00000
S.Kentucky	5	3		6,7667		6,7667±,025166
Klep.P	5	3		6,7667		6,7667±0,25166
S. Enteritis	5	3		7,1000		7,1000±,020000
E.Faecalis	5	3		7,1000		7,1000±0,20000
E.Aerogens	5	3		7,1000		7,1000±0,20000
S.Infantis	5	3		7,1000		7,1000±0,20000
S.Typhim	5	3		7,1000		7,1000±0,20000
S. Enteritis	15	3		7,5000		7,5000±0,50000
E.Faecium	5	3		7,5000		7,5000±0,50000
E.Faecalis	15	3		7,5000		7,5000±0,50000
S.Typhim	15	3		7,5000		7,5000±0,50000
E.Aerogens	15	3		7,5000		7,5000±0,50000
S.Infantis	15	3		7,5000		7,5000±0,50000
S.Kentucky	15	3		7,5000		7,5000±0,50000
Klep.P	15	3		7,5000		7,5000±0,50000
E.Faecium	15	3			8,5000	8,5000±0,50000
Pseud.flo	5	3			8,5667	8,5667±0,45092
Sig	.		1,000	,064	,837	
Total						1,80747

Means for groups in homogeneous subsets are displayed.
a- Uses Harmonic Mean Sample Size = 3,000.



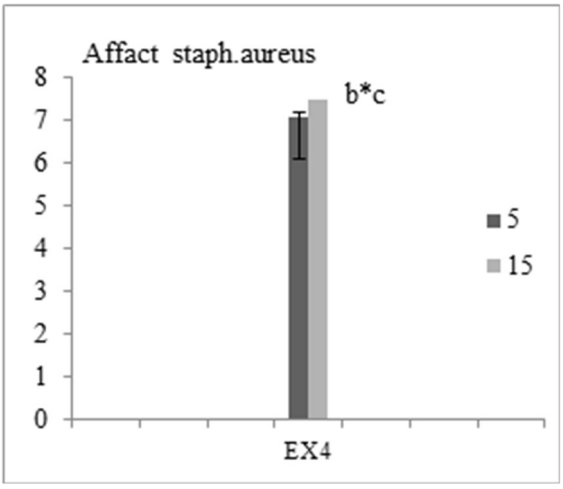
Graphic 3.1. Affect the extracts on S.enteritis.

As Graphic the Papaver somniferum was in 15 concentration higher than 5 cons and Prunus amygulus where recorded $8.5\pm0.5^{b\bullet c}$ and in 5 cons $=7.5\pm 0.5$ where was similar 15 cons in P. Amygdulus while the lowest degree was in 5 cons of Prunus amygdlus 7.1 ± 0.215^{b}.



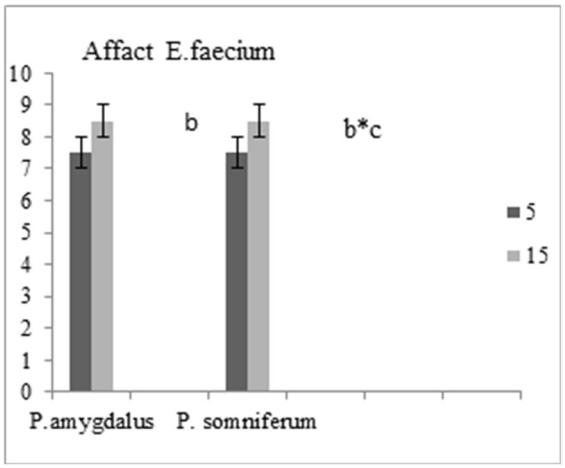
Graphic 3.2. Affects the extracts on C.albicanis.

Just Papavar somniferum have an effect, but 15con was higher than 5 con whereas recorded in 15 cons and 5cons $7.1\pm0.1^{b\bullet c}$, 2.0 ± 3.2 respectively.



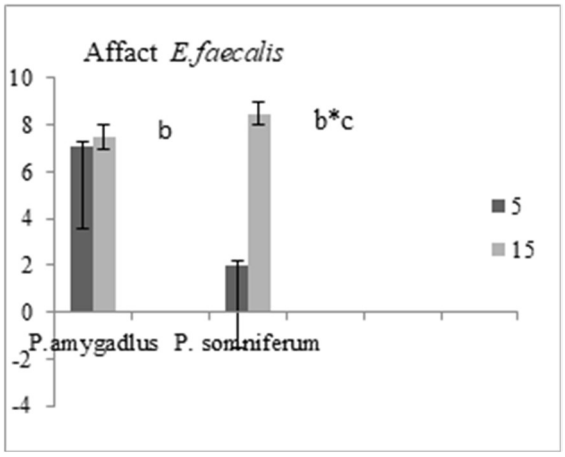
Graphic 3.3. Affect the extracts on Staph. aureus.

Just Papaver somniferum 15 cons higher than 5 cons where was $7.5\pm0.5^{b\bullet c}$ while in 5 cons was $7.1\pm0.1^{b\bullet c}$.



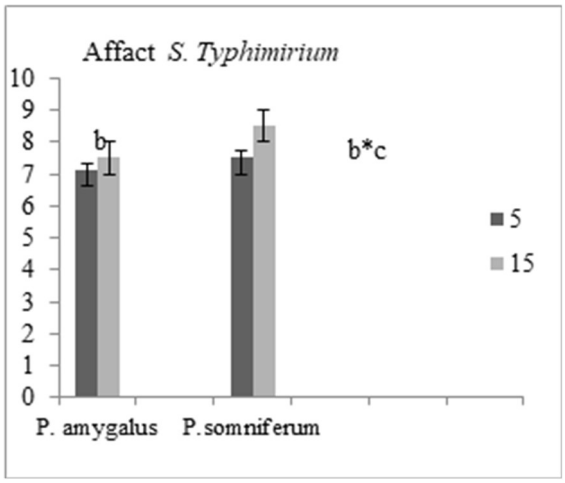
Graphic 3.4. Affect the extracts on E.faecium.

P- Amygdulus and P. Somniferum have similar results in 15 cons and 5 cons 8.5±0.5, 7.5±0.5, respectively.



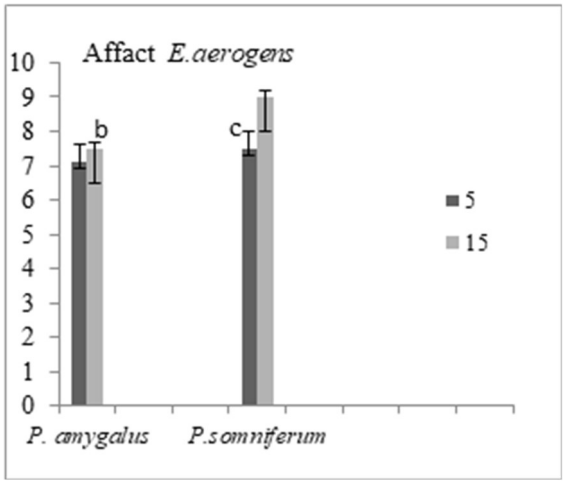
Graphic 3.5. Affect the extracts on E. faecalis.

In P. smygdulus was 5 cons higher than 5 cons in P. somniferum 7.1±0.2^{b}, 2.0±3.5 respectively. While in 15con of P. somniferun was the highest about 8.5±0.5$^{b•c}$.



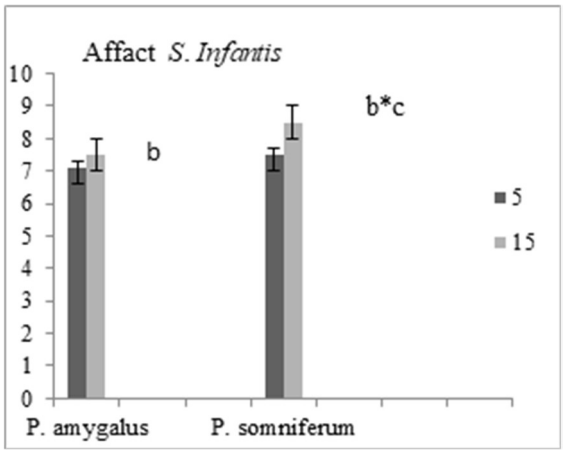
Graphic 3.6. Affect the extracts on S. typhimirium.

P- somniferum noted the highest results represent in 15 cons about 8.5±0.5$^{b•c}$ while the lowest one noted in 5con of P. amygdlus about 7.1±0.2^{b}.



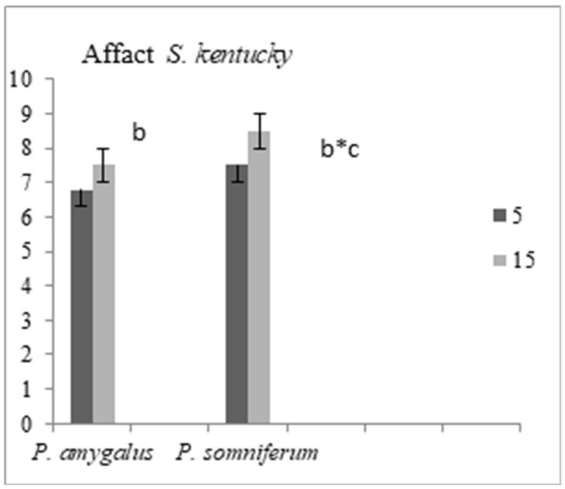
Graphic 3.7. Affect the oil on the E.aerogens.

The highest degree noted in 15 cons of P. somniferum where was 9.0±1.0^{c} while the lowest degree in 5 cons of P. amygdulus about 7.1±0.2^{b}.



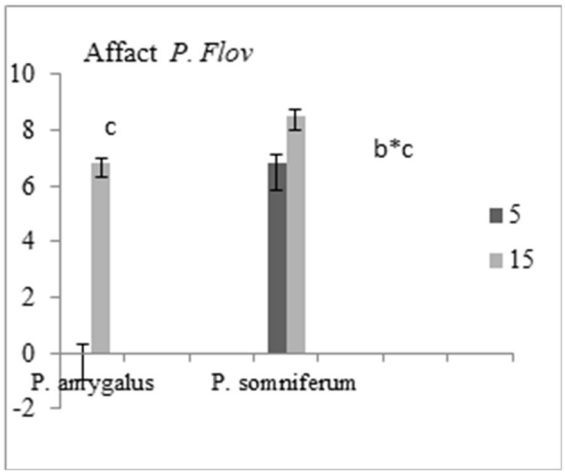
Graphic 3.8. Affect The Extracts On S.Infantis.

In 15 cons of P. somniferum was 8.5±0.5$^{b•c}$ while in P. amygdulus 7.5±0.5 and the lowest one was in 5 cons about 7.1±0.2^{b}.



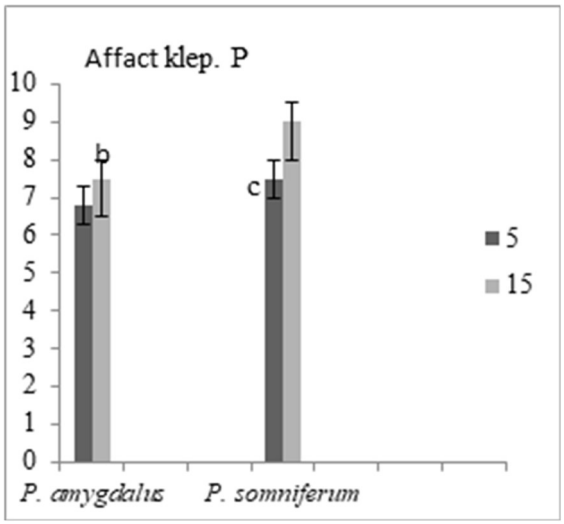
Graphic 3.9. Affect The Extracts On S.Kentucky.

The highest significance was in 15 con of P. Somniferum about 8.5±0.5$^{b•c}$ while the lowest one was in 5 con of P. Amygdulus about 7.1±0.2^{b}.



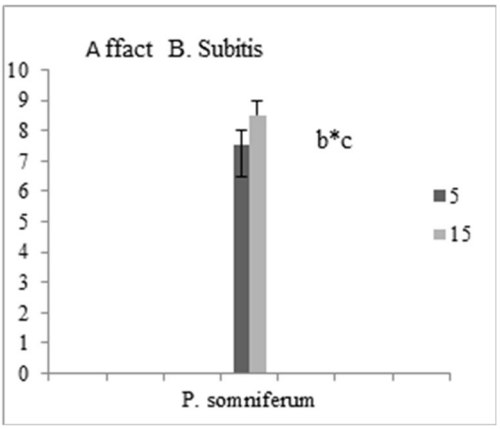
Graphic 3.10. Affect The Extracts On P.Floverse.

As shown in Graphic the highest result was in 15 cons of P. Somniferum while 5cons was equal with 15 cons P. Amygdulus about 8.5±0.5. whereas no signifance in 5 cons of P. Amygdulus.



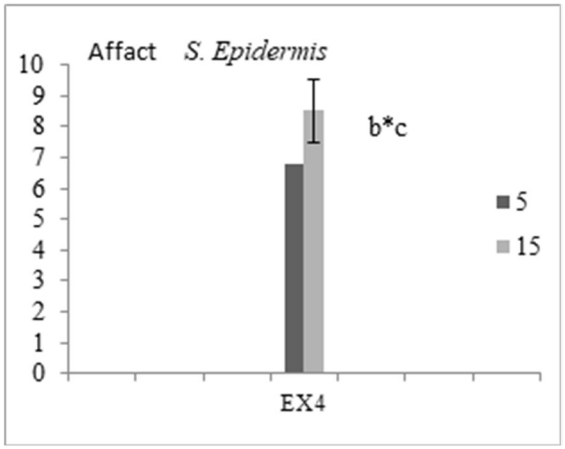
Graphic 3.11. Affect The Extracts On Kleb.Pneumonia.

The P. somniferum was the highest significanc about 9.0±1.0^{c} and both extracts was similar affect in 5 cons in P. somniferum and 15 cons in P. amygdlus about 7.5±0.5^{b}.



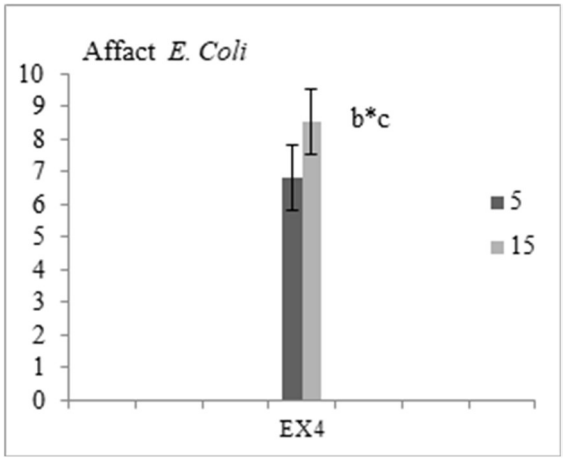
Graphic 3.12. Affect The Extracts On B.subitis.

P- somniferum in 15 cons 8.5±0.5$^{b\bullet c}$ was higer than 5 cons 7.5±0.5$^{b\bullet c}$.



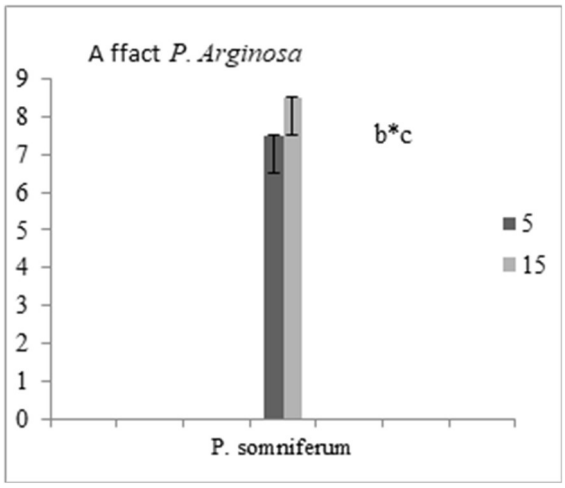
Graphic 3.13. Affect the extracts on S.epidermis.

P- somniferum in 5 con =6.7±0.2^{a} lower than 15 cons 8.5±0.5$^{b\bullet c}$.



Graphic 3.14. Affect the extracts on E.coli.

Ex4 =Papaver somniferum in 5 con =6.7±0.2$^{b\bullet c}$ lower than 15cons 8.5±0.5$^{b\bullet c}$.



Graphic 3.15. Affect the extracts on P.arginosa.

Black Papaver somniferum in 5 cons = 7.5±0.5$^{b\bullet c}$ lower than 15 cons 8.5±0.5$^{b\bullet c}$.

Antibacterial activities of S. indicum, white P. somniferum and Nigella sativa result showed no zone inhibition at all as (Photo 3.4). While N. sativa there is a little effect against E.aerogens, S. infantis, S. enteritis as shown in (Photo 4.5) in my opinion due to a low of the concentrations did uses.

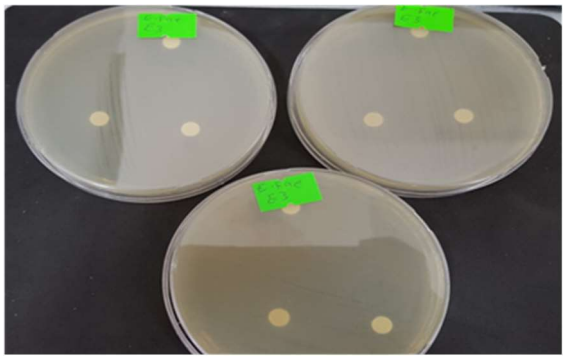


Photo 3.4. Anti-microbial activities of S. indicum.

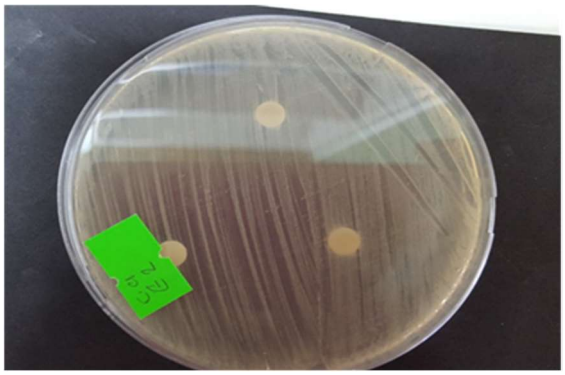
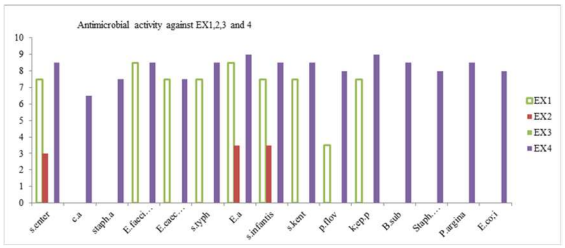


Photo 3.5. Anti-fungal activity of N. Sativa.

Black P. Somniferum has effected on all microbial, the opposite of the results of Sesame, White P. Somniferum no affected. N. Sativa mild affected on S. enterlids, E. aerogenes and S. infantis. P. amygdulus affected on both of S. enterlids, E. faecium, E. aerogenes, E. faecilis, S. typhimurim, S. kentucky, P. flov, klep. P and S. Infantis. As (Graphic 3.16).



Graphıc 3.16. Antimicrobial activity against EX1,2,3 and 4

*** Minimal Inhibition Concentration Method**

The result in minimal inhibition concentration method as in (Table 3.6) whereas minimal concentration in black Papaver somniferum was constant with fourteen microbial 12.5µ/ml except on Candida albicanis and Staph. aureus 25µ/ml as showm (Photo 3.9) while in Prunus amygdalus oil was 12.5µ/ml in E. asrogens, E. faecium and 25µ/ml in S. enteritis, E. faecalis, S. typhimirium, E. asrogens, S. infantis, S. Kentucky, Pseudomonas floverscans, Klebsella pneumonia as shown (Photo 3.7) and lastly with Nigella sativa was 100µ/ml in both S. enteritis, Staph. aureus, E. faecalis as shown (Photo 4.8).

Photo 3.7. Prunus amygdalus MICS.

Photo 3.8. Nigella Sativa MICS.

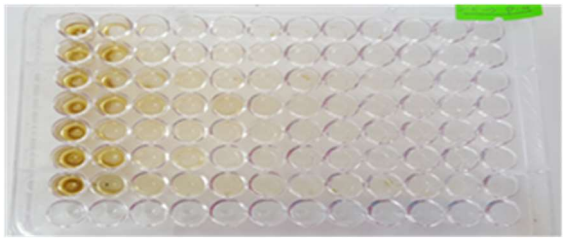


Photo 3.9. Papaver Somniferum MICS.

Table 3.6. Minimum Inhibitory Concentration Results /µ/mL.

Minimum inhibitory concentration Results µ/ml			
	Prunuus amygdalus	*Nigella sativa*	*Papaver somniferum*
S. enteritis	25	100	12.5
Candida albicanis	-	-	25
Staph. aureus	-		25
E. faecium	12.5	-	12.5
E. faecalis	25		12.5
S. typhimirium	25	-	12.5
E. aerogens	12.5	100	12.5
S. infantis	25	100	12.5
S. Kentucky	25	-	12.5
Pseudomonas floverscans	25	-	12.5
Klebsella pneumonia	25	-	12.5
B. subitis	-	-	12.5
Staph. epidermis	-	-	12.5
E.coli	-	-	12.5
Pseudomonas arginosa	-	-	12.5

reported poppy seeds failed to inhibit any of the tested bacteria that seed pushed of Pakistan, then boiling 10g in 100ml distilled water and used disc diffusion method [40]. While Prunus amygdalus has affected against some of the bacteria the highest result showed against Pseudo. flore in 15µ/disc concs about 8,5±0,4 and on E. facium about 8,5±0,5 while reporting with Neogi et al (2008) that collect seeds of the rainy season has affected where was the highest inhibition zone against Salmonella typhi of Gram nagtive 20±0.9 and peniclium notaum of fungi 20±0.9 were used well diffusion method for antimicrobial test and essential oil [39], but with another study by used well disc diffusion and leaves has no inhibition zone against S. aeures and E. coli. However, in white P. somniferum , sesame and N. sativa have no affected on microbes whereas reported with Shittu et al (2007) who used the essential oil that extracted from sesame leaves has a very strong antimicrobial effect against Streptococcus pneumoniae at full concentration while strong and mild antimicrobial effect on Candida albicans [41]. Chaudhry et al (2008) studies conducted on N. sativa that seeds bought from Pakistan reported good result against Staphylococcus aureus 19.6 ± 1.8, but no affected on pseud. aergenisa and klep. P [40]. In addition, with Grasas et al (2005) reported N. sativa oil affected against twenty-four of bacteria by using the agar diffusion method [65)

*** Summary**

The summary of this research that tested antimicrobial activity of medical seeds oil, noticed there are the antimicrobial activity of black P. somniferum and P. amygdalus oil that had used, while of N. sativa, S. indicum, P. samniferum (white) not affected on the microbe, but does not mean it is not good because the results depend on many factors:

a- The concentration
b- Antimicrobial test
c- Extracted oil
d- The region and season
e- Leaves or seeds.

This study has revealed also that seeds are a rich source of: many nutrients and fatty acid by Gas chromatography–mass spectrometry (GC-MS) analysis that appears to have a very positive effect on human health do not have any side effects.

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