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# Evaluation of antimicrobial activity of linseed ( *Linum usitatisimum* ) on different types of bacteria

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## Abstract:

Linseed has different benefits that must be carried toward improvement of quality of life of humans .To reduce the dose dependent side effects and resistance by an antibiotic, there are many alternative approaches nowadays ,one of these approaches is using linseeds as antimicrobial agent by using different form of linseeds like powder ,oil and ethanol extract ,and evaluate their antimicrobial activity using different strains of bacteria such as *Staphylococcus Sp., Bacillus Sp., Escherichia Coli , Proteus Sp., Klebsiella Sp. , Streptococcus Sp., and Pseudomonas Sp.* From the results, it was concluded that linseeds have significant antimicrobial activity on these strains which depends on storage condition, extraction methods and concentration used.



## Keywords:

Flaxseed, linseed, lignin's, antimicrobial activity, Secoisolariciresinol diglucoside (SDG), flaxseed extract.


## Objectives:

1. Examine the antimicrobial activity of flaxseed & linseed oil against different species of bacteria.
2. Find which linseed form is responsible for antimicrobial activity.
3. Introduce flaxseed as an effective drug in the future.

## Introduction

Flax (Linum usitatissimum) (1) (2), also known as common flax or linseed, is a member of the genus Linum in the family Linaceae (3). It is an upright annual plant growing to 1.2 m (3 ft. 11 in) tall, with slender stems. The leaves are glaucous green, slender lanceolate, 20–40 mm long and 3 mm broad. The flowers are pure pale blue, 15-25 mm diameter, with five petals; they can also be bright red. The fruit is a round, dry capsule 5-9 mm diameter, containing several glossy brown seeds shaped like an apple seed, 4-7 mm long ( figure 1 ) (4).

Flax flower

Flax dry capsule

Apple like shape seeds

The medicinal parts are the stem as a sterile linen thread, the oil extracted from the seeds, the dry ripe seeds, the linseed cakes and the fresh flowering plant. (5, 6,7)
Characteristics: The plant flowers only in the morning.
Habitat: The plant is cultivated in temperate and tropical regions. (5).
More than 200 species are present in the genus *Linum* including cultivated specie *L.usitatissimum*.The oil is known as linseed oil .
Flaxseeds occur in two basic varieties:

1- Brown
2- Yellow or golden.

( 4)
Figure ( 2 )

Whole flaxseeds are chemically stable, but ground flaxseed may go rancid when left exposed to air at room temperature in as little as one week because of oxidation. Refrigeration and storage in sealed containers will keep ground flax from becoming rancid for a longer period. If packed immediately without exposure to air and light, milled flax is stable against excessive oxidation when stored for nine months at room temperature and under warehouse conditions, for twenty months at ambient temperature. The lignans (secoisolariciresinol (SDG)) which are

responsible for antimicrobial activity of flaxseeds are a large group of polyphenols can be metabolized to the mammalian lignans (enterodiol and enterolactone) by human intestinal micro flora. Secoisolariciresinol diglucoside (SDG), the major flaxseed lignan, is 75-800 times more abundant in flaxseeds than in other food sources Along with its derivative (secoisolariciresinol (SECO)) (8).

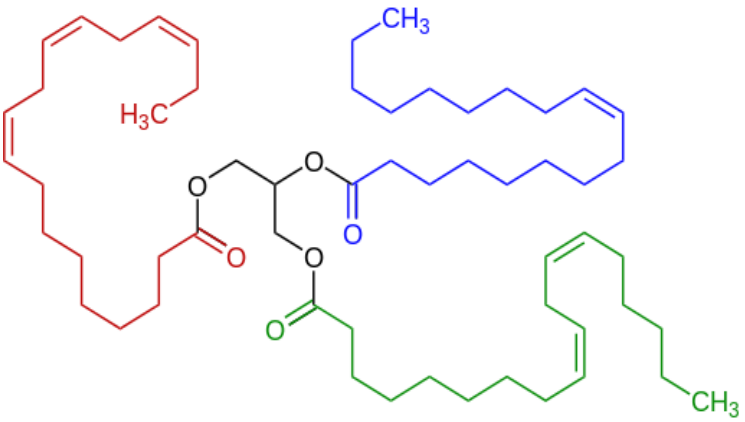



Figure 3 : flaxseed chemical structure .
Chemical representation for triglyceride content (Linolenic Acid $C_{18}H_{30}O_2$ Linoleic Acid $C_{18}H_{32}O_2$ Oleic Acid $C_{18}H_{34}O_2$ ) (9)

## Active ingredients:

Linolenic acid(40-70%), Linoleic acid(10-25%), Alpha-linolenic acid and Oleic acid(13-30%) are all fatty acids. Galactose, Xylose, Arabinose, and Rhamnose are mucilage, Equol is a protein flavonoid(6,10)

This mixture of flavonoids, phenolic acids and lignans is proposed to be more effective antibacterial agent than pure ,single compound.

The flavonoids present in flaxseeds inhibit DNA synthesis in *Proteus* sp. or RNA synthesis in *Staphylococcus* sp.(11, 12).

Consumption of flaxseed has been demonstrated to have multitude positive health benefits including decreasing rate of tumor growth, reducing serum cholesterol level and decreasing incidence of breast, prostrate, and colon cancers(13)(14)

## Nutritional values per 100g (15) :

| | |
|---|---|
| Energy | 2,234 kJ (534 kcal) |
| Carbohydrates | 28.88 g |
| Sugars | 1.55 g |
| Dietary fiber(SDG) | 27.3 g |
| Fat | 42.16 g |
| Saturated | 3.663 g |
| Monounsaturated | 7.527 g |
| Polyunsaturated | 28.730 g |
| omega‑3 | 22.8 g |
| omega‑6 | 5.9 g |
| Protein | 18.29 g |
| Vitamins | |
| Thiamine (B1) | (143%) 1.644 mg |
| Riboflavin (B2) | (13%) 0.161 mg |
| Niacin (B3) | (21%) 3.08 mg |
| Pantothenic acid (B5) | (20%) 0.985 mg |
| Vitamin B6 | (36%) 0.473 mg |
| Vitamin C | (1%) 0.6 mg |
| Minerals | |
| Calcium | (26%) 255 mg |
| Iron | (44%) 5.73 mg |
| Magnesium | (110%) 392 mg |
| Phosphorus | (92%) 642 mg |
| Potassium | (17%) 813 mg |
| Zinc | (46%) 4.34 mg |

## Effects :

In animal experiments a reduction of cholesterol levels in the liver was observed (due to the unsaturated fatty acids). A blood sugar lowering effect was also proven. The anti tumer effect is attributed to the lignans (lignans are anti-mycotic, anti-oxidative and anti-estrogenic)(6,7,16).

Dosage Forms: Capsules — 1000 mg, 1300 mg, oil, powder, soft gel capsules

## Daily Dosage:

Constipation — l dessertspoon of whole or bruised (not ground) seed with at least 150 ml of liquid 2 to 3 times daily.

-Lower Cholesterol — 35 to 50 gm daily of the crushed seeds. May be incorporated into muffins or breads (17).

-Decrease platelet aggregation — l to 2 tables poon full flaxseed oil daily(18)

-Gastritis and enteritis

To prepare a demulcent for use in gastritis and enteritis, allow 5 to 10 gm of whole seeds to stand in cold water for 20 to 30 minutes, then pour off the liquid and take 2 to 4 tablespoons of milled linseed (the seeds should not be taken in the dry state, should be pre-hydrated) (19)

## materials &Methods :

Sample: Samples of brown seeded flaxseed *(Linum usitatissimum* ) were obtained from a local store in ashar ,basrah , iraq

Microbial cultures: microorganisms were obtained from biology department of science college .

The following microorganisms were used for the study:

| bacteria tested | source of specimen |
|---|---|
| *staphylococcus sp.* | Wound |
| *streptococcus sp.* | Skin |
| *E . coli* | wound , burn |
| *klebsiella sp.* | Urine |
| *Proteus sp.* | Wounds |
| *Bacillus sp.* | Wound |
| *Pseudomonas sp.* | Wound |

Media :mueller-hinton agar

Method: Disc diffusion susceptibility method

## Procedure :

### Preparation of powder solution :

1. seeds was grounded and fine powder obtained .
2. 0.5 gm of powder dissolved in 10 ml DMSO(Di methyl Sulfoxide) solvent for 30 minutes.

DSMO: an organic water miscible highly polar reagent with high boiling point used because it doesn't effect on antimicrobial activity of flaxseed .

### Method of linseed extraction :

### Preparation of Defatted Flaxseed Powder

Flaxseeds were ground in a grinder to obtain a fine powder (110–120 mesh). The powder was defatted with n-hexane (1: 6 w/v) at room temperature for 16 h. The defatted powder was air dried for 18 h and stored in deepfreeze (– 20 °C) for further use(10).

### Lignans extraction procedure:

The defatted powder of flaxseed cultivars (200 g) was blended with absolute ethanol 100% (1.2 L) at 30 °C for 24 hours, The solvent concentration, the extraction temperature, and the extraction time were set according to the requirement of the experiment.. The extract was filtered using sand core funnel then Extracts were concentrated to dryness under vacuum at 45°C, using a rotary evaporator at 90 rpm. Light yellow syrup was obtained. The syrup was hydrolyzed with 1M NaOH at room temperature for 16 h .The hydrolyzed syrup was acidified with 0.5M HCl to pH 6 using a pH meter .The solution was cooled down to 15°C then centrifuged with a high-speed centrifuge at 2000 rpm for 10 min to precipitate and remove water soluble polysaccharides and proteins after filtering the salt with a sand core funnel The extracts were weighed to calculate the yield and were dissolved in small volume of initial solvent and stored at –18°C, until tested(20).

## Preparation of oil extract :

1- Filter paper discs were prepared and sterilized by UV sterilizer for 30 minutes .

2- Sterilized discs put in linseeds oil and sterilized together by UV sterilizer for 30 minutes.

## Preparation of culture medium : using Mueller – Hinton agar

1- ( 28g ) of agar powder were dissolved in ( 1 )liter of water and sterilized by UV sterilizer device .
2- Poured on Petri dish ( we ensured that the opening of the beaker is sterilized by burner ) , let it cold at room temperature .
3- Using streak plate method , the inoculum bacteria was streaked on agar surface ( that were activated and inoculated in Mueller – Hinton agar) by using streaking loop and burner for sterilization .

## Preparation of linseeds powder medium :

a. two holes were made in the solid agar ( first one 0.5 cm diameter and the second one 0.25 cm diameter ) .
b. the solution of linseeds powder was poured .
c. Media plates were incubated at 37°C for 18 to 24 h in an incubator
d. the inhibition zone diameter was observed and measured by metered ruler.

## Preparation of linseeds oil medium :

Method 1 : the discs put ( impregnated in oil ) on agar surface directly and overnight inoculate then the inhibition zone was observed & measured .

Method 2 : holes of 0.25 cm in diameter are made and added the oil by yuing micro pipette , inoculated overnight and then the inhibition zone observed and measured by metered ruller

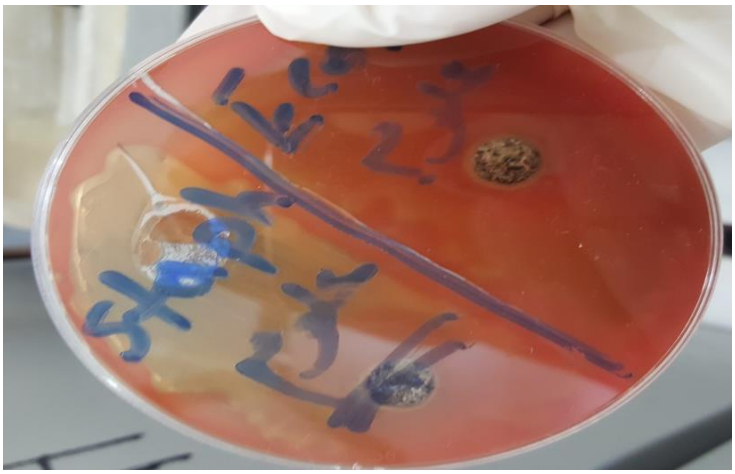
## Preparation of linseed ethanol extract medium :

1- holes of 0.25cm in diameter were made and linseed extract was added by micropipette .
2- The petri dishes were incubated in the incubator overnight .
3- Inhibition zone measured by metered ruler.

# Results and discussion

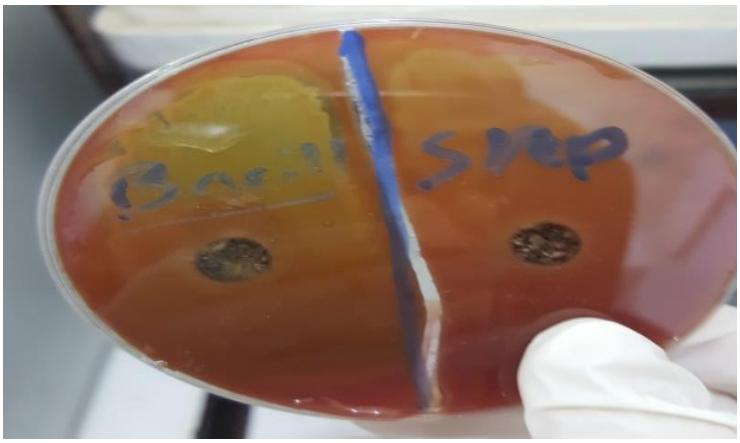
## 1- Linseed powder solution

| Bacteria | Diameter of Inhibition zone (mm) |
|---|---|
| *staphylococcus sp.* | 10 |
| *streptococcus sp.* | 12 |
| *E . coli* | 13(wounds),11(burns) |
| *klebsiella sp.* | 13 |
| *Proteus sp.* | 13 |
| *Bacillus sp.* | 14 |
| *Pseudomonas sp.* | 12 |



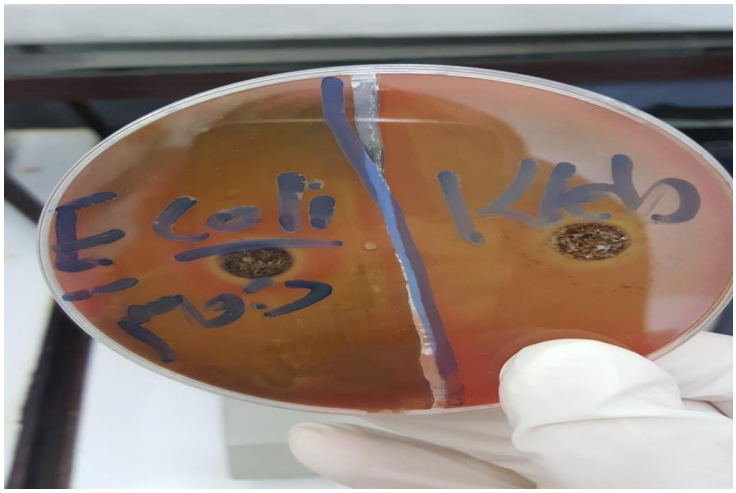
Staphylococcus sp. inhibition zone on the left

E. coli inhibition zone on the right



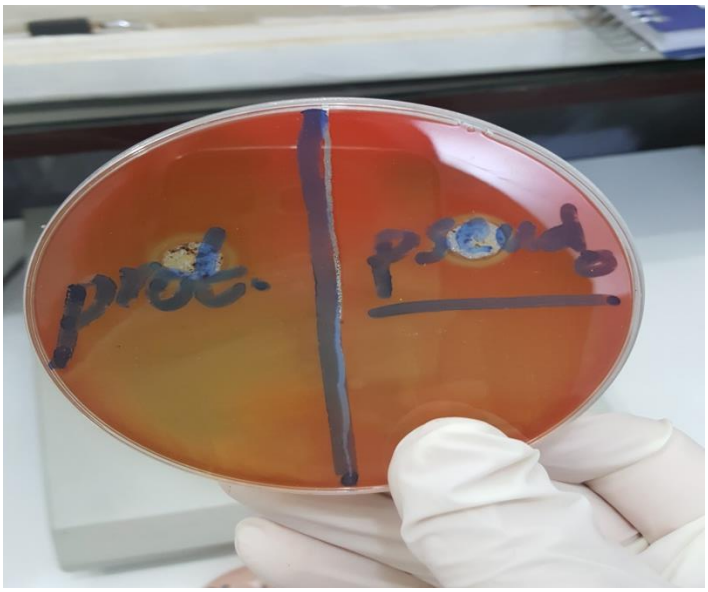
Bacillus sp. Inhibition zone on the left.

Streptococcus sp. inhibition zone on the right .




E.coli (was taken from another source) inhibition zone on the left

Klebsiella sp. Inhibition zone on the right




Proteus sp. Inhibition zone on the left .

Pseudomonas sp. Inhibition zone on the right

Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition around the powder(21) ,Maximum antimicrobial activity was observed against gram +ve bacteria, The antibacterial activity profile of flaxseed against tested bacteria indicated that E. coli , Bacillus , proteus & klebsiella sp. was the most susceptible bacterium(22). The presence of lignans may bind both Ca+2 and Mg+2, thereby reducing the Ca+2 and Mg+2 from lipopolysaccharide of the outer membrane causing a release of lipopolysaccharide, thereby destabilizing the membrane, which may increase the activity of lignans (5).

## **linseed oil** :

| Type of bacteria | Concentration | results |
|---|---|---|
| *E.coli* | 0.5 ml ,0.25 ml | Growth ( negative) |
| *Staphylococcus sp.* | 0.5ml , 0.25 ml | Negative |
| *Proteus sp.* | 0.5 ml , 0.25 ml | Negative |
| *Bacillus sp.* | 0.5 ml , 0.25 ml | Negative |
| *Klebsiella sp.* | 0.5 ml , 0.25 ml | Negative |

Figure ( 4)

As it is illustrated in the results table that linseeds oil have negative results on that four types of bacteria . This results are opposite to (1) study that show a significant antimicrobial activity as a different concentrations were used **0.25, 0.5, 1.0, 2.0** ml and their results were as following (1) .

| S. No | Dose (ml) | Selected Microbes (Zone Diameter in mm) | | | |
|---|---|---|---|---|---|
| | | *Bacillus subtillus* | *Staphylococcus aureus* | *Escherichia coli* | *Shigella boyedi* |
| 1. | 0.25 | 1.00 | 3.67±0.58 | 0.00 | 0.00 |
| 2. | 0.5 | 2.33±0.58 | 4.33±0.58 | 1.00 | 1.33±0.58 |
| 3. | 1.0 | 4.00 | 5.67±0.58 | 2.33±0.58 | 3.00 |
| 4. | 2.0 | 7.33±0.58 | 6.67±0.58 | 4.67±0.58 | 5.33±0.58 |

* All values are in mm units and value = mean ± s.d.

A very important factor is the content of both extracted total polyphenols and lignans , both of them cause low stability which mean they undergo oxidative rancidity in which the unsaturated fatty acid react with oxygen to form peroxides . The peroxide decompose to yield a complex of mixture including aldehyde , ketones and other volatile product which cause an odor and flavor . The other stability problem is hydrolytic rancidity , temperature that control the rate of fat oxidation . linseed oil produce by conventional presser machines that generate heat that effect the linseed stability by accelerating the oxidation process which result in decomposition of the content responsible for antimicrobial activity leading to negative antimicrobial activity .There are many approaches to improve linseed stability as general such as improvement in storage stability can be gained by lowering the storage temperature and the use of packaging materials with low oxygen permeability. (23)

Oxidation problem can be solved according to (United state department of agriculture USDA by adding of synthetic phenolic antioxidants used in foods and cosmetics – including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertbutylhydroquinone (TBHQ) – with natural phenolic antioxidants extracted from plants. (23)(24) .

Other method is to use cold presser technique (as mentioned previously in linseed oil ethanol extraction procedure ) which produce a cold-pressed oil containing about 78 percent of carbon, 11 percent of hydrogen, and 11 percent of oxygen; while the hot-pressed oil contains nearly 3 percent less carbon, and nearly 3 percent more oxygen . (24)

## linseed ethanol extract

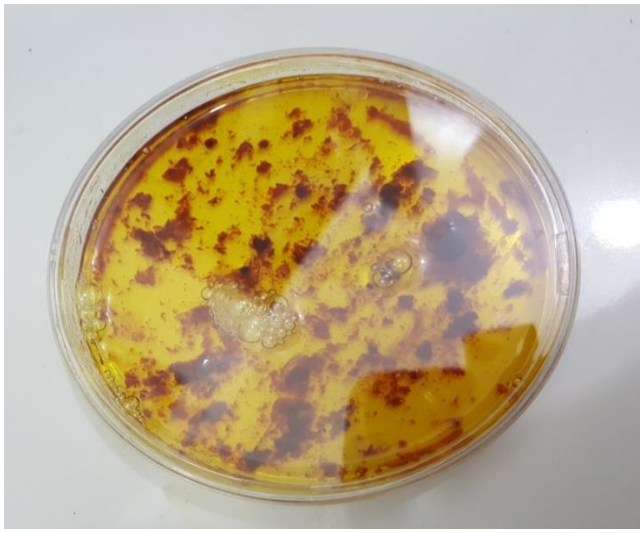
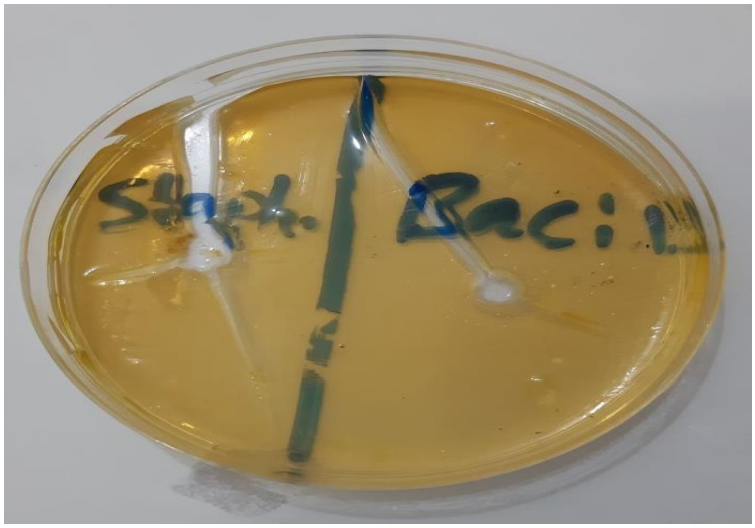


Figure ( 5)

| Bacteria | Diameter of Inhibition zone (mm) |
|---|---|
| staphylococcus sp. | 27mm |
| Bacillus sp. | 40 mm |
| klebsiella sp. | Negative results |
| Proteus sp. | 30mm |


Klebsiella sp. on the left

Proteus sp. on the right




Staphylococcus sp. on the left

Bacillus sp. on the right

The response of each microorganism tested was different. Lignans extract revealed antimicrobial activity showing different selectivity for each microorganism . Lignans extract was found to be the most effective antibacterial against the Gram positive bacteria such as *Bacillus sp*. compared to Gram negative bacteria such as *Klebsiella sp*. ,which was the most resistant bacteria to lignans extract. The activity of lignans extract might be due to their ability to combine with bacterial cell wall and ,therefore ; inhibiting the microbial growth. The presence of lignans may bind both Ca+2 and Mg+2, thereby reducing the Ca+2 and Mg+2 from lipopolysaccharide of the outer membrane causing a release of lipopolysaccharide, thereby destabilizing the membrane, which may increase the activity of lignans(5). From the tables it is quite evident that ethanolic extract of flaxseeds are effective against, *Staphylococcus sp.& bacillus sp*. , but did not show any antimicrobial activity against *Klebsiella sp*. these result are in agreement with(25) research study .

## conclusion :

linseed has significant antimicrobial activity on different strains of bacteria . The antimicrobial activity differ according to the linseed form used . Linseed powder yield a good antimicrobial activity and linseed ethanol extract gave excellent antimicrobial activity with large inhibition zone of each of four types bacteria tested while no inhibition of growth with linseed oil produced by heat presser machine which might happened due to loss of stability by rancidity and hydrolysis .Stability can be improved by many techniques such as using cold presser extraction method , storage in low temperature in the refrigerator and at closed ,oxygen impermeable systems. An acceptable dosage forms for administration of linseed are opaque hard gelatin capsule , soft gelatin capsule , big container for powder contain tight closure with cotton and silica gel packet used to control the moisture and humidity. They are basically made from silicon dioxide that works efficiently in protecting the food products and pharmaceutical tablets and capsules from moisture inside the packaging box, bottle or container.

## References :


1. Joshi Y, Garg R , Juyal D . Evaluation Of Synergistic Antimicrobial Activity Of Gemifloxacin With Linum Usitatissimum Seed Oil , The Journal Of Phytopharmacology 2014; 3(6): 384-388 .
2. Tawheed Amin, Monika Thakur. A Comparative Study On Proximate Composition, Phytochemical Screening, Antioxidant And Antimicrobial Activities Of Linum Usitatisimum L. (Flaxseeds),International Journal Of Current Microbiology & Applied Science Issn: 2319-7706 Volume 3 Number 4 (2014) Pp. 465-481
3. Ganorkar &Jain, oil health benefit 2015
4. Quanru Liu; Lihua Zhou. "Linum". Flora Of China. 11. Archived From The. "Linum Usitatissimum". Germplasm Resources Information Network (Grin). Agricultural Research Service (Ars), United States Department Of Agriculture (Usda). Original On 5 April 2015
5. Cowan Mm, Plant Products As Antimicrobial Agents. Clin Microbiol Rev, 12, 1999, 564–582.
6. Allman Ma, Pena Mm & Pang D: Supplementation With Flaxseed Oil Versus Sunflower Seed Oil In Healthy Young Men Consuming A Low Fat Diet: Effects On Platelet Composition And Function. In: Eur J Clin Nutr 49(3): 169-178, 1995.
7. Joerg Gruenwald, Phd Thomas Brendler, Ba.Pdrfor Herbal Medicines Version Sr-21. 2015.
8. Andeera .I.Pag,Dana.J.Radu,Flaxseed Cake– A Sustainable Source Of Antioxidant And Antimicrobial Extract ,2011
9. Arthur H. Church , Chemical Structure Of Linseed Oil . The Chemistry Of Paints And Paintings , 23 -01- 2011.
10. Hosseinian et. al. "Nutrition Facts For 100 G Of Flaxseeds". Conde Nast For Usda National Nutrient Database,. Archived From The Original On 2010-12-05.
11. Rehwald A et.al., Hplc Analysis Of The Flavonoids Of Crataegi Folium Cum Flore. In: Pm 59(7)28. 1993.
12. Gaafar Aa, Salama Za, Askar Ms, El-Hariri Dm, Bakry Ba.. In Vitro Antioxidant And Antimicrobial Activities Of Lignan Flax Seed Extract (Linum Usitatissimum, L.). Int. J.Pharm. Sci. Rev. Res. 47: 291-297. 2013.
13. Hosseinian Fs, Et.Al. , Aaph-Mediated Antioxidant Reactions Of Secoisolariciresinol And Sdg, Org Biomolchem, 5, 2007.

14. Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M "Flax And Flaxseed Oil: An Ancient Medicine & Modern Functional Food". Journal Of Food Science And Technology. 51(2014).
15. "Nutrition Facts For 100 G Of Flaxseeds". Conde Nast For Usda National Nutrient Database, Version Sr-21. 2015. Archived From The Original On 12-05-2010 .
16. Miller Lg, Herbal Medicinals: Selected Clinical Considerations Focusing On Known Or Potential Drug-Herb Interactions. Arch Intern Med; 158:2200-2211,1998 .
17. Arjmandi Bh, Khan Da. Juma S Et Al., Whole Flaxseed Consumption Lowers Serum Ldl-Cholesterol And Lipoprotein(A)Concentrations In Postmenopausal Women. In: Nutr Res18(7): 1203-1214, 1998.
18. Anonym, Pharmaceutical Care:'"Den Mifjbrauch Von Laxanzien Vermeiden Helfen." In: Daz 135(20): 1867-1868. 1995.
19. Bisset Ng & Wichtl M (Eds): Lini Semen. Herbal Drugs And Phytopharmaceuticals: A Handbook For Practice On A Scientific Basis. Medpharm Scientific Publishers, Crc Press, Stuttgart,Germany, Pp 298-300. 1994.
20. Zhang Zhen-Shan, Dong Li, Li-Jun Wang, Necatiozkanc, Xiao Dong Chena, Zhi-Huai Mao, Hong-Zhi Yang. Optimization Of Ethanol–Water Extraction Of Lignans From Flaxseed, Sep Puriftechnol, 57, , 17 – 24 , 2007 .
21. Jehan Bakht1, Huma Ali. Antimicrobial Activities Of Different Solvents Extracted Samples Of Linum Usitatissimum By Disc Diffusion Method, African Journal Of Biotechnology 10(85), Pp. 19825-19835, 28 December, 2011.
22. Gezer K, Duru Me, Kivrak I, Turkoglu A, Mercan N, Turkoglu H, Gulcan S, Free-Radical Scavenging Capacity And Antimicrobial Activity Of Wild Edible Mushroom From Turkey, Afr J Biotechno, 5, 2005, 1924–1928.
23. Andreea I. Pag . Flaxseed Cake – A Sustainable Source Of Antioxidant And Antibacterial Extracts, Cellulose Chemistry And Technology,March 19, 2013 .
24. Dr. Joseph G. Sebranek, Iowa State University, And Dr. Stephen Neel, World Food Logistics Organization , Racidity And Antioxidant , Revised 2008 .
25. Alaa A, et.al. , Invitro Antioxidant And Antimicrobial Activities Of Lignin Flax Seed Extract. Int.J.Pharm .Sci,Rev,Res.,23( 2) ,Dec 2013 .

**جمهورية العراق**
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# اختبار فعالية نبات الكتان كمضاد بكتيري على أنواع مختلفة من البكتريا

**أعداد**

**ولاء أسعد عبدالله** **مروة منذر هومان**

**أشراف :**

**د. قاسم فوزي عبد الكريم** **د. زينب صبيح وريوش**

8 أيار 2018

# نبذة مختصرة:

بذور الكتان لها فوائد عديدة يجب استغلالها لتحسين صحة الكائن الحي، ولتقليل الآثار الجانبية الناتجة عن استخدام المضادات الحيوية الكيميائية ولتقليل المقاومة ضد المضادات الحيوية . هناك العديد من الطرق البديلة في الوقت الحاضر ، أحد هذه البدائل هي استخدام بذور الكتان كعامل مضاد للميكروبات باستخدام عدة اشكال مثل مسحوق البذور وزيت بذور الكتان ومستخلص الإيثانول ، وتقييم نشاطها المضاد للميكروبات باستخدام سلالات واصناف مختلفة من البكتيريا مثل المكورات العنقوديه،العصويات، الاشريكيه القولونيه ،المتقلبة ،الكلبسيلة ،العقدية و البكتيريا الزائفة، من خلال نتائج العمل تم الاستنتاج أن بذور الكتان لها نشاط مضاد للميكروبات على هذه السلالات والذي يعتمد على طريقة التخزين المادة وتركيزها وطرق استخلاصها .