

The effect of hot water extract of *Linum usitalissimum* and *Trigonella foenum-graecum* on bacteria that isolated from mouth

Mohammed A AL-Jaleel, Anmar Saadi Aboud and Alyaa Muhsin Yousif

Department of Biology, College of science, AL-Mustansiriyah University, Iraq.

Abstract

Research was done in Laboratories of biology department-collage of check antibacterial activity of hot water extract of flax seed (*Linum usitalissimum*) and fenugreek seed (*Trigonella foenum-graecum*) against bacteria that isolated from mouth by using holes method on nutrient agar.

Extracts was prepared of seeds of plants that studying in stoke then bacteria that isolated from mouth of different people in different age categories and do different habits such as (drinking wine ,un brush teeth regularly). Some species of bacteria were diagnosis in this experiment which were (*pseudomonas spp.* *Staphelococcus* and *Streptococcus spp.*) this experiment were measured the diameter of inhibition zone around the holes in petri dishes. Both extracts of *Linum* and *Trigonella* seed showed an antibacterial activity against bacteria in study, also the *Trigonella* seed extract present more effective than *Linum* seed extract on bacteria.

Keywords: *Linum*; *Trigonella*; Water Extract; Antibacterial

1. Introduction

In recent years, studies on medicinal plants have increased, as they are considered an important source of many drugs, medicines and other medical materials from ancient times to the present. People use them to treat many diseases because they contain secondary metabolites [1].

1.1. Fenugreek (*Trigonella foenum-graecum*)

- Kingdom: Plantae
- Division: Magnoliophyta Class: Magnoliopsida
- Order: Fabales
- Family: Fabaceae
- Genus: *Trigonella*
- Species: *T. foenum-graecum*. [2]

The fenugreek (*Trigonellafoenum-graecum*) belongs to the family Fabaceae and is a multiuse and commercially important spice crop grown for its seeds, tender shoots, and fresh leaves. It is an annual plant, extensively cultivated as a food crop in India, the Mediterranean region. North Africa, and Yemen. Fenugreek seeds and herbs are well known for their distinct aroma and slightly bitter taste. The cultivation of this crop is conned to areas with moderate or low rainfall and a cool growing season without extreme temperatures. It can tolerate 10-15°C frost. [3], it has been reported to be

* Corresponding author: Anmar Saadi Aboud
Department of Biology, College of science, AL-Mustansiriyah University, Iraq.

grown under a wide range of soil and climatic conditions in many countries of Europe, Asia, Africa, Australia, and America Fenugreek has economic value as food, fodder, medicine, and in cosmetics.

According to the natural medicines comprehensive database, 523 commercial products containing fenugreek have been reported, including 50 Canadian licensed products [4].

1.1.1. Chemical constituents

- Alkaloids: Trimethylamine, Neurin, Trigonelline, Choline, Gentianine, Carpaine .and Betain
- Amino acids: Isoleucine, 4-Hydroxyisoleucine, Histidine, Leucine, lysine, L- tryptophan, Argenine.
- Saponins: Graecunins, fenugrin 18, fenugreek, trigofenosides A-G. Steroidal sapinogens: yamogenin diosgenin, smilagenin, sarsasapogenin, tigogenin, neotigogenin, gitogenin, neogitogenin, yuccageninaponaretin. Flavonoids: Quercetintinetixin isovetixin.
- Fibers: Gum, neutral detergent, fiber
- Other: Coumarin, lipids, vitamins, minerals 28%. [5]

1.1.2. Pharmacological Uses

- Antidiabetic activity
- Antiplasmodic activity
- Hypolipidemic activity
- Immunological activity
- Antibacterial activity
- Anthelmintic activity
- Antioxidants activity. [7]

1.2. Flax (*Linum usitalissimum*)

- Kingdom : Plantae
- Subkingdom : Tracheobionta
- Superdivision : spermatophyta
- Division : Magnoliophyta
- Class : Magnoliopsida
- Subclass: Rosidae
- Order : Linales
- Family: Linaceae
- Genum: Linum
- Species: usitalissimum

Is a member of the genus *Linum* (flax) in the family Linaceae. It is a food and fiber crop cultivated in color regions of the world. The textiles made from *Linum* are known in the Western countries as linen, and traditionally used for bed sheets, underclothes, and table linen. The oil is known as linseed oil. In addition to referring to the plant itself, the word "flax" may refer to the unspun fibers of the flax plant. The plant species is known only as a cultivated plant, and appears to have been domesticated just once from the wild species *Linum bienne*, called pale flax. [8,9]

1.2.1. Chemical constituents

- Glycosides
- Alkaloids
- Flavonoids
- Saponins
- Resins
- Protein (22.2%)
- Oil (45.19%)
- Carbohydrates (28.09%). [10]

1.2.2. Pharmacological Uses

Antidiarrheal activity analgesic activity immunological activity reduce danger of cancer Antioxidant activity it used as laxative reduce danger cancer.[11].

2. Materials and methods

We took dried seeds of two mentioned plants (*Linum usitatissimum* and *Trigonella foenum-graecum*) then we grinded them.

Then we added 10g of powder of both (*Linum usitatissimum* and *Trigonella foenum-graecum*) to 300ml of boiled distill water in conical flask and close it tightly and leave in for 24 hours.

In the next day we filtrate the solution of the extract that we prepared

We get clear extract

Then we pour extract of both mentioned plants in flat dishes (such as petri dish) and let them until dry.

After we have dry extract we have to scrape it to have powder extract [12].

Then 2 concentration were prepared which were (0.5% & 1%) from both extracts.

2.1. Preparation of nutrient agar media

8.4g of Nutrient agar powder is weighted and dissolve in 300ml of distal water (2.8g per 100ml) then sterile the solvent by autoclave for 20 minute at 121 centigrade after 20 minutes we let it cooled then pour it in petri dishes there we have ready media to culture samples [13].

2.2. Culture samples

We took sample swabs from mouth of people in different categories (male or female) some of them drinking wine and all samples were UN regularly teeth brush. As shown in Table (1).

Table 1 Samples taken from mouth

No.	Age	Male or female	Drinking wine or not	Diagnose	notes
1	22	Male	Yes	Staphylococcus & streptococcus	None
2	37	Female	No	Staphylococcus	Drinking wine
3	26	Male	Yes	streptococcus	
4	25	Male	No	Pseudomonas	
5	36	Male	No	Staphylococcus	Drinking wine
6	32	Male	No	No growth	
7	40	Female	No	streptococcus	Drinking wine
8	22	Male	No	Staphylococcus& fungi	
9	22	Male	No	Pseudomonas	
10	21	Female	No	Staphylococcus	
11	22	Male	No	Staphylococcus& fungi	
12	40	Male	No	Staphylococcus& fungi	Drinking wine
13	38	Female	No	streptococcus	
14	22	Male	No	Streptococcus& fungi	
15	36	Male	Yes	Pseudomonas	

The isolated bacteria appears in cultured samples were diagnosis according to some researchers[14,15]

2.3. Test inhibition activity of extracts

Bacterial samples are used to check their sensitivity to hot water extract.

Prepare nutrient agar then take swab from taken samples and make holes on dishes. on the plate (by adding the prepared concentration into the hole 0.5% and 1%) and inoculate with extract until the hole fill with extract then incubate the samples for 18-20 hours to see result.

3. Results and discussion

3.1. Bacterial diagnosis

Three genus of bacteria were diagnosis in this experiment which

- Pseudomonas spp.
- Staphelococcus spp.
- Streptococcus spp.
- Fungi.

3.2. *Linum* seeds

After preparing the isolates and placing the extracts in the dish as described above in the methods of work and after incubation for 18-24 hours, the effect of *Linum* seeds extract on the bacterial isolates shown.

The results are as shown in the table (2), the hot aqueous extract appear an active role to prevent growth the bacteria, these

Table 2 Inhibition zones of *linum* hot extract against bacteria by (mm)

Sample	Inhibition zone (mm)	
	Concentration 0.5%	Concentration 1%
1	2	4
2	3	5.5
3	2.5	5
4	3.5	6
5	4	6
6	2	4
7	3	5
8	3.6	6.5
9	2.1	4
10	4.2	7
11	4	8
12	3	5
13	4	7
14	2	4
15	3	6

Results due to the compounds were in extract which causes the death of bacteria around the holes in petri disc. These results were agree with another studies which aims to an antibacterial effect on oral pathogens bacteria cultured on nutrient agar such (*Pseudomonas spp.* And *Streptococcus spp.*). (Almad, et.al.2018) & (Son & Song .2017).

Also the results in this experiment agree with Zuk, et.al. [17] which showed the hot extract components had antibacterial activities, and were useful as a preventative against bacterial infection.

3.3. *T. foenum-graecum* Extract

After preparing the isolates and placing the extracts in the dish as described above in the methods of work and after incubation for 18-24 hours, the effect of the *Trigonella* seed extract on the bacterial isolates is shown in table (3).

Table 3 Inhibition zones of *Trigonella* hot extract against bacteria by (mm)

Sample	Inhibition zone (mm)	
	Concentration 0.5%	Concentration 1%
1	3	6
2	4	8
3	5	9
4	6	11
5	5	10
6	5	11
7	4	9
8	3	6
9	6	10
10	6	12
11	3	7
12	3	6
13	4	10
14	2	5
15	5	10

The results in this experiment appears an active role for hot aqueous of *Trigonella* on bacteria under study, the compounds in extract showed an obvious antibacterial effect on bacteria.

These results were agree with other studies [15] which mentioned that the hot aqueous extract showed an antibacterial activity against six isolate of bacteria (*pseudomonas*, *E.coli*, *salmonella*, *staphelococcus*, *klebseilla*, *Shigella*). Also agree with Walli R., et.al. [18] which showed the hot aqueous extract of fenugreek seed had antimicrobial effect against G⁺, G⁻ & fungi.

Recommendation

We recommended that adding an extracts of *Linum* and *Trigonella* seeds to too paste will be useful for preventing or reducing the growth of pathogenic bacteria in mouth.

4. Conclusion

The results illustrated an active role for hot aqueous of *Trigonella* on bacteria and the compounds in extract showed an obvious antibacterial effect on bacteria.

Compliance with ethical standards

Acknowledgments

The author thanks warmly the College of science, AL-Mustansiriyah University, Iraq.

Disclosure of conflict of interest

Authors declared that there was no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

- [1] Allaby RG, Peterson GW, Merriwether DA, Fu YB. Evidence of the domestication history of flax (*Linum usitatissimum* L.) from genetic diversity of the *sad2* locus. *Theor Appl Genet*. 2005 Dec;112(1):58-65. doi: 10.1007/s00122-005-0103-3. Epub 2005 Oct 8. PMID: 16215731.
- [2] Almad BEM, A.B.. Kashmoola, Muhannacl MAi, etal. The antibacterial effect of Flax seed extract on selective oral pathogens-comparative *in vitro* study. 2018. 7(11):1-11.
- [3] Alwhibi Mona S, Solimen Dina A. Evalueting the antibactenical activity of fenugreek (*trigonella foenum-graccum*) seed extract against asclection of different pathogenic bacteria. *J. pure X Appliedc Micribio*. 2014; 8(2): 817-821.
- [4] American Public Health Association. Standard methods of water analysis, 1992.
- [5] Brown AE. Bensons HJ. Benson's microbiological applications : laboratory manual in general microbiology. New York, N.Y. : McGraw Hill Education, 2015.
- [6] Dave Kelly. Towards a numerical definition for divaricate (interlaced small- leaved) shrubs. *New Zealand Journal of Botany*. 1994; 32(4): 509.
- [7] Habh, A. M.M., Idan, R. M., Deli H. F., Study the effect of some fungal isolates that treated by crude oil on seed germination Celery (*Apium graveolens*). *Journal of Al-Mustansiriah Sciences*, 2015, 26(1): 1-5.
- [8] Hussien ZG, Aziz RA. Chemical composition and antibacterial activity of *Linum Usitatissimum* C. (Flaxseed). *SYS. Rev. Pharm*. 2021; 12(2): 145-147.
- [9] Habh AMM, Khalil MAA, Rami MI, Sabah R. Increase the readiness of phosphorus to raphanus salivus & *Vigna unguicalata* by local isolates of bacteria *pseudomonas* spp. *Iraqi J. of Biotech*. 2-15,14(2): 90-99.
- [10] Kim Su Ji. et al.Hypoglycemic and antihyperlipidemic effect of fenugreek seeds in alloxan induced diabetic rats. *American journal of biochemistry and biotechnology*. 2006; 2(4): 154-160.
- [11] Mashadian NV, Rakhshandeh H. Antibacterial and antifungal effects of *Nigella sativa* extracts against *S. aureus*, *P.aeroginosa* and *C. albicans*. *Pak J Med Sci*. 2005; 47-52.
- [12] Lawn RJ, Imrie BC. Crop improvement for tropical and subtropical Australia. *Designing plants for difficult climates. Field Crops Research*.1991, 26(2):113-139.
- [13] Prasad K. Reduction of serum cholesterol and hypercholessorolemic in rabbits by SDG isolated from flaxseed. *Circulation*. 1999; 99(10): 1355-1362.
- [14] Vyas S, et al. Analgesic and inflammatory activity of *Trigonella foenum- graecum* seeds extract, *Pubmedcentral. Acta Poloniae pharmaceutica –Drug Research*. 2008; 65(4): 473-476.
- [15] Son, hyeon-Jeong, Song KB. Antimicrobicel activity of Flax seed meal extract against *E.coli* 0157: H7 and *Staphylococcus aureus* inoculated on red mustard. *J.Microbical. Biotechnol*. 2017; 27(1): 67-71.
- [16] Vanisree M, Lee CY, Lo S, Nalawae SM, Lin C, Tsag HS. Studies on the production of some important selonaly Meta botiteis medical by plant tissue Cartui. *Bot Ball Acad*. 2003; 451-22.

- [17] Zuk M, et. al.. Bacterial activities of G.M flax seed cake extract on pathogenic bacteria clinical strains. BMC Biotechnol. 2014; 14: 70.
- [18] Walli, Rhab R, et.al. Screening of antimicrobial activity of fenugreek seeds. Pharmacy & pharmacology International J. 2015; 2(4): 122-124.