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# Antibacterial Activity of Flaxseeds Extracts on Streptococcus mutans: *In Vitro* Study

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## ABSTRACT

*Natural products including plants had been used as a source for many antimicrobial components, now there is increasing interest for developing such components because of being non-chemical and non-synthetic. Flaxseeds have been long used in traditional medicine as it has antimicrobial effect. The present study tended to examine the antimicrobial effect of flaxseeds oil and aqueous extracts on Streptococcus mutans in comparison with chlorhexidine. For this purpose, flaxseeds extracts was first processed; then ten iterations was done for agar diffusion test in which wells was loaded with the test agents and diameter of inhibition zones was calculated, and then ten iterations was done for testing the effect of flaxseeds extracts on the acidogencity and adherence of Streptococcus mutans on extracted teeth surface. The results showed that both flaxseeds extracts (oil and aqueous) exhibited antibacterial activity against Streptococcus mutans, showed an increase in this activity as the concentration of extracts increased and also showed effectiveness in inhibition the adherence and acidogencity of Streptococcus mutans. In conclusion, flaxseeds extracts can be an effective approach for dental caries prevention.*

**Key words:** Antimicrobial effect, Flaxseed, *Streptococcus mutans*, Adherence, Acidogencity

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## INTRODUCTION

Dental caries consider one of the frequent occurring health problems in the world. For years, many antimicrobial agents had been used in an attempt to control growth of bacteria with the increasing in utilization and misuse of antimicrobial agents, many bacteria had produce strains that resist antimicrobial agent, leading to difficulties in controlling them. Natural products including plants had been used as source for many antimicrobial components [1].

Flaxseeds are the seeds of flax plant, that has fibrous stem; considerable branches; slender leaves; flowers white to blue in color with seeds inside capsules and branched root [2,3]. The flaxseeds shape is oval and one end pointed, texture is smooth shiny surface, color is yellow to dark brown depending on the amount of pigment that found in the outer coat of the seed [4].

Flaxseeds composed of dietary fibers (soluble, insoluble and phenolic compounds), proteins (unsaturated fatty acid and saturated fatty acids) proteins (some essential and non-essentials amino acids). Also contain vitamin like Vitamin E and vitamin K, minerals like calcium, magnesium, phosphorus and high amount of potassium [4]. Flaxseeds have antimicrobial effect on some types of microorganisms due to some of the its active compounds like phenolic compounds, long-chain unsaturated fatty acids. For example Mustafa et al. demonstrated that the aqueous extract of flaxseeds had antifungal activity against candida albicans greater than the used Nystatin and it may be attributed to the presence of phenolic content that cause disrupting of cell wall and deconstructing of protein. Also found that the flaxseeds oil had no antifungal activity against candida albicans [5].

Flaxseed effect had been studies also on other oral condition like periodontitis for example antimicrobial effect of ethanolic flaxseed extract

had been tested against Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Tannerella forsythia, founding bacteriostatic effect on these microorganism [6]. flaxseed activity had been tested by Altaei et al. on oral ulcer using flaxseeds oil paint and demonstrated decreasing in the time to regenerate mucosal tissue and pain duration without adverse effects [7].

## MATERIALS AND METHODS

### Saliva collection

Stimulated saliva samples were collected under standardized conditions from ten healthy-looking persons (18-30 years old) without history of systemic diseases from dental specialize center in Babil, to obtain ten *Streptococcus mutans* isolates.

### Isolation of microorganisms

10-fold serial dilutions for the collected saliva samples were prepared using sterile normal saline. Inoculate dilutions 10-3 on Sucrose- Bacitracin Agar (SB20) incubated first anaerobically for 48 hrs. at 37°C then aerobically incubated within 37°C for 24 hrs [8].

### Identification of microorganisms

*Streptococcus mutans* identification was based on morphology of the culture colony under dissecting microscope, morphology of the microbial cells using gram stain, carbohydrate fermentation test, catalase production test and Vitek 2 compact system [9].

### Preparation of flaxseeds extracts

Flaxseeds were purchased from the scientific Maeshab Al-Alhikma a local herbal market in Al-Hilla city, Babylon governorate, Iraq, gently rinsed with distilled water (D.W), air dried at room temperature and stored at cool dry place in paper bag till used.

### Aqueous extract

Following the method that used by Al-Shawi et al (2017) with some modifications, grashing 1000g of flaxseeds using mortar and pestle and mixed with 3 L D.W left on rotary shaker at 10 rotation\ min for 2 days, then centrifuge at 4000 rpm for 5 min with discarding the residue filtrated, drying in laboratory drying oven [10].

### Oil extract

Using mechanical oil presser (Grains YDZY), flaxseeds placed in the seeds feeder, and as the machine operated the compressing process of the seeds forcing the oil to escape through perforated section then collected in a bottle, put in refrigerator [11].

Stock solution was prepare in 10 mg\ml concentration from each extract by mixing the dry aqueous extract with D.W and the oil with Dimethylsulfoxide (DMSO) and D.W using vortex mixer and ultrasound apparatus and then 5 different concentrations were prepared from it: 62.5, 125, 250, 500 and 1000 mcg/ml (Figure 1).

### *In vitro* experiments

### Antibacterial activity

Agar diffusion test had been used in this study to assess the antibacterial activity of the flaxseeds extracts on *Streptococcus mutans*. loading the wells that had been made on Muller Hinton agar plates with 100 µl of each concentration of flaxseeds extract, chlorhexidine at 0.2%, distilled water where included as positive and negative controls, respectively. with including solvent that use in the stock preparation, after inoculated with 0.1ml of a 24 hrs. Activated

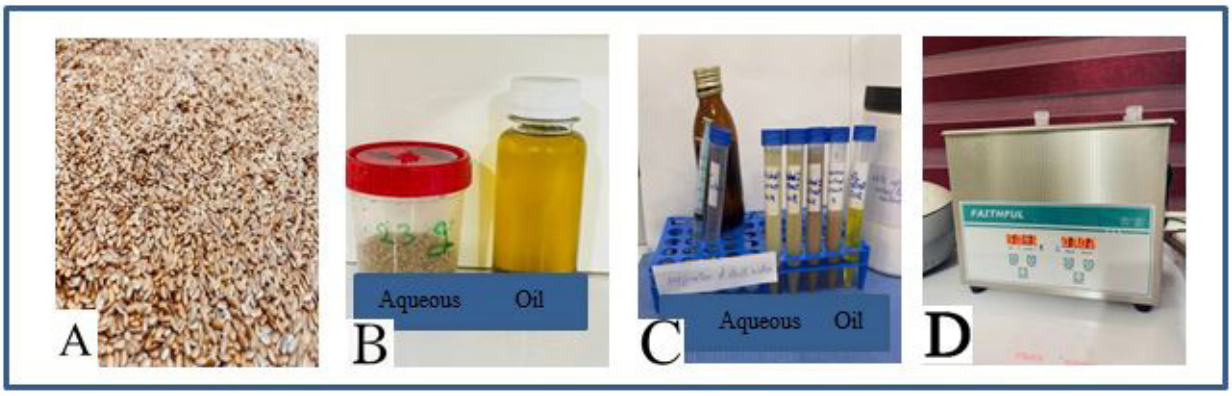


**Figure 1: A-flax seeds; B-aqueous extract and oil extract; C-flaxseeds aqueous and oil stock solutions. D-flaxseeds stock solutions in the ultrasonic machine.**

broth culture of *Streptococcus mutans*. Incubated the plate aerobically for 24 hrs. at 37 C , follow by measuring the diameter of growth inhibition zone [12].

**Adherence of *Streptococcus mutans***

Sound extracted teeth had been used in this study to assess the effect of the flaxseeds oil and aqueous extracts on adherence of *Streptococcus mutans*. Teeth were cleaned and polished with non-fluoridated pumice and stainless steel wire had been bind around the root of each tooth and sterilized. Prepare 7 tube containing 10 ml of sterile brain heart infusion broth with 5% sucrose. Each tooth and stainless wire was immersed in different tested agents (flaxseeds extracts at concentrations of 125,250, 500 mcg/ml, CHX at 0.2% and distilled water) for 2 min then rinse with normal saline and left to dry at room temperature. The teeth and stainless wires were immersed in the prepare tubes and inoculated with 0.2 ml of activated bacterial isolates (except control negative) and incubated aerobically for 7 days at 37 C. Plaque formation detection done by using dental probe in which founding of plaque was indication for failure of the tested agent to prevent Mutans streptococci adherence [13] (Figure 2).

As mentioned in adherence experiment, teeth, stainless wires and the 7 tubes were prepare and incubated aerobically at 37 C for 3 days with transferring all wires that binding the teeth every 24 hrs. into a newly prepare 0.05 sucrose broth, incubated within 37o C. After that in the 4th day immersing the teeth in 10 ml of each tested agents individually (flaxseeds extracts at concentrations of 125,250, 500 mcg/ml, CHX at 0.2% and distilled water) was done for about 2 min and then rinsed with normal saline and left to dry at room temperature. All the teeth were placed in a fresh 5% sucrose broth containing 1% bromocresol purple as an indicator for bacterial acid formation. Then, the tubes incubated within 37o C for 1 weeks. Acid production was indicated through the changing of the colour of pH indicator from purple (remaining purple in color meaning that the agent was efficient in prohibiting acid formation) to yellow (when the agent was not effective in preventing acidogenicity) Or to orange (when the agent was weak and partly effective in preventing acidogenicity) [14].

## RESULTS

The tests of this research was performed in ten replications .As demonstrated in the Table 1, both of the Flaxseeds extracts (aqueous and oil) were effective in inhibition *Streptococcus mutans* growth. Inhibition of *Streptococcus mutans* growth appeared as clear zone around the wells that filled with flaxseeds extracts at most concentrations except the smallest

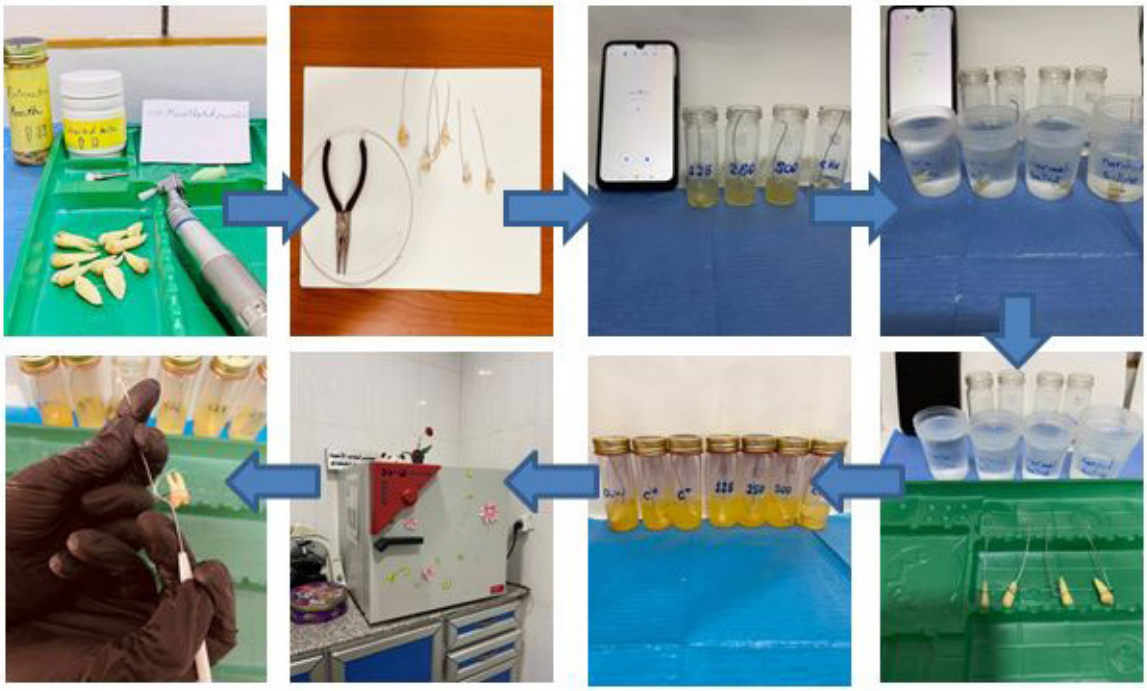

**Figure 2: Adherence of *Streptococcus mutans* test. Acidogenicity of *Streptococcus mutans***

**Table 1: Antibacterial activity of different concentrations of flaxseeds extracts against *Streptococcus mutans*.**

| Extract | Conc. | Mean | ±SD | F | P value | ES |
|---|---|---|---|---|---|---|
| Oil | 62.5mcg | 0 | 0 | 292.091 | 0.000* | 0.839 |
| | 125mcg | 13.6 | 1.174 | | | |
| | 250mcg | 15.2 | 1.033 | | | |
| | 500mcg | 16.7 | 1.337 | | | |
| | 1000mcg | 20 | 2.211 | | | |
| Aqueous | 62.5mcg | 0 | 0 | 311.636 | 0.000* | 0.847 |
| | 125mcg | 0 | 0 | | | |
| | 250mcg | 10.4 | 1.506 | | | |
| | 500mcg | 14.2 | 1.619 | | | |
| | 1000mcg | 17 | 1.7 | | | |

^=not significant at p >0.05, * =significant at p <0.05

**Table 2: Comparisons of diameter inhibition zone of S. Mutans among each extract with CHX using Dunnett t (2-sided).**

| Conc. | (I) Interaction | (J) Interaction | p value |
|---|---|---|---|
| 62.5 | Oil | CHX | 0 |
| | Aqueous | CHX | 0 |
| 125 | Oil | CHX | 1 |
| | Aqueous | CHX | 0 |
| 250 | Oil | CHX | 0.01 |
| | Aqueous | CHX | 0 |
| 500 | Oil | CHX | 0 |
| | Aqueous | CHX | 0.95 |
| 1000 | Oil | CHX | 0 |
| | Aqueous | CHX | 0 |

**Table 3: Effect of different concentration of flaxseeds extracts on *Streptococcus mutans* acidogenicity.**

| Extract | Colors in each concentration | | | Fisher exact | Total |
|---|---|---|---|---|---|
| Oil | 125mcg | 250mcg | 500mcg | | 21purple |
| | 3purple7orange | 8purple | 10purple | 0.002* | 9orange |
| Aqueous | 10yellow | 6orange | 8purple | 0.000* | 8purple |
| | | 4yellow | 2orange | | 8orange |
| | | | | | 14yellow |
| Total | 3purple | 8purple | 18purple | | 29purple |
| | 7orange | 8orange | 2orange | | 17orange |
| | 10yellow | 4yellow | | | 14yellow |

**Table 4: Effect of different concentration of flaxseeds extracts on *Streptococcus mutans* adherence.**

| Extract | Colors in each concentration | | | Fisher exact | Total |
|---|---|---|---|---|---|
| | 125mcg | 250mcg | 500mcg | | |
| Oil | 10present | 9present | 8present | 0.753^ | 27present |
| | | 1absent | 2absent | | 3absent |
| Aqueous | 10present | 8present | 6present | 0.129^ | 24present |
| | | 2absent | 4absent | | 6absent |
| Total | 20present | 17present | 14present | | 51present |
| | | 3absent | 6absent | | 9absent |

^=not significant at p>0.05, *=significant at p<0.05

concentration that did not show inhibition zone for the two extract in addition to concentration 125 mcg/ml in aqueous flaxseeds extract did not show inhibition zone. *Streptococcus mutans* were sensitive to flaxseeds oil at concentrations 125, 250, 500 and 1000 mcg/ml, and not sensitive at 62.5 mcg/ml. *Streptococcus mutans* were sensitive to flaxseeds aqueous extract at concentrations 250 ,500 and 1000 mcg/ml, and not sensitive at 62.5 and 125 mcg/ml. There was increase in inhibition zone diameter with the increase in the extracts concentration. *Streptococcus mutans* were more sensitive to flaxseeds oil than to aqueous extract. At high concentrations, *Streptococcus mutans* were sensitive to flaxseeds extracts more than to chlorhexidine (showing larger inhibition zone diameter) (Table 2).

The ability of flaxseeds extracts to inhibit the adherence and acidogencity of *Streptococcus mutans* increased with the increase of extracts concentrations; 500 mcg/ml was the most effective between the tested concentrations (Tables 3 and Table 4).

## DISCUSSION

With the expansion of antibiotics resistance, neoteric types of antimicrobial substances are promptly required. So medicinal plants extracts play an important role in the pharmaceutical industry as it regarded as a premium alternative to the usual antimicrobial drugs with no or minimum side effects [15]. This study demonstrated antibacterial activity and growth inhibition capability of flax seeds oil extracts against *Streptococcus mutans* which came in agreement with the findings of Yusoff et al. [16] in addition to demonstrated that flax seeds aqueous extract had antibacterial activity and growth inhibition capability. The activity of flaxseeds extracts could be ascribed to the presence of some active compounds that act alone or in combination to inhibit *Streptococcus mutans* growth. For example Flaxseed oil contains fats, flavonoids, glycosides, phenols and tannins according to Joshi et al [17]. In particular flaxseed has plenty of secoisolariciresinoldiglucoside (SDG) which consider the precursor of lignans and could be responsible about the antibacterial activity in the different flaxseeds extracts [18]. In this research flaxseeds oil extract demonstrated higher growth inhibitory activity against S. Mutans than the aqueous extract. The flaxseeds aqueous and oil extracts also demonstrated inhibition of acidogencity of *Streptococcus mutans* which might be due to effecting of the enzymes which are necessary for carbohydrate fermentation and acidogenicity. Flaxseeds oil being more effective in activity against S. Mutans than the aqueous extract. Both flaxseeds extracts demonstrated inhibition of adherence of *Streptococcus mutans* and show dependent on the concentration of the extract which might be due to effecting the glucosyl transferase enzyme (Gtf) activity which consider important part in formation of insoluble glucan and considered an essential of plaque and adherence of S. Mutans as suggested by Weli et al. for ginger extract [19].

## CONCLUSION

Flaxseeds extracts had revealed antibacterial activity against *Streptococcus mutans*; therefor it can be used as an effective oral health preparation that helps in prevention dental caries.

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