

## Original Research Article

### Antibacterial activity of flax seeds extract rinse against *streptococcus mutans* colonies

Comment [L1]: uppercase

#### Abstract:

**Background:** Flax seeds has health-potentiating medicinal benefits due to the presence of a remarkable amount of bioactive compounds. The antibacterial activity of ethanolic extract of flax seeds against *B. cerues*, *S. aureus*, *Ps. Aeruginosa* and *K. pneumoniae* has been documented. Therefore, the study current study aims to investigated the antibacterial activity flax seeds extract rinse against *streptococcus mutans* colonies

Comment [L2]: First time appearing in the text, write full name: *Bacillus cereus*

Comment [L3]: First time appearing in the text, write full name: *Staphylococcus aureus*

Comment [L4]: *Pseudomonas aeruginosa*

Comment [L5]: First time appearing in the text, write full name: *Klebsiella pneumoniae*

Comment [L6]: the current study

Comment [L7]: uppercase

Comment [L8]: delete

Comment [L9]: lowercase

**Methodology:** It was a preclinical experimental study and sample size was  $n = 60$ . The participants were given an envelope for group randomization. Film of plaque from labial surface of teeth of study participants was collected on sterile strips that was transported to laboratory for culture in sterile containers. For culture *S. mutant* samples were inoculated in Columbia Agar. The flax seeds extract extract was diluted in distilled water in 1:4 (Extract: Distilled water) concentration. And participants were divided into 3 groups. (negative control, positive control, flax seed extract group). Diluted flax seed extract was given to experimental group for rinses, distilled water was given to negative control group and Positive controls were given a commercially available mouth rinse. Next sample of plaque was collected after two hours to observe the effects of flax seed extract on bacterial colonies.

**Results:** The growth of colonies was calculated on growth media plates in samples prior to rinsing and samples that were taken after the rinsing. The pre and post samples showed significant ( $p$ -value  $< 0.05$ ) decrease in number of colonies in positive control group (conventional rinse) and flax seed extract group.

**Conclusion:** Flax seeds extract mouth rinse reduced the number of colonies equally as that of commercially available mouth rinse.

**Key words:** Antibacterial activity, flax seeds extract, streptococcus mutans.

Comment [L10]: avoid words that are already in the title

#### Introduction:

Comment [L11]: remove underlining and colon

Flax seeds or *Linum usitatissimum*, are commonly known as linseed and are known as *Alsi* in Pakistan and India (1). It is

one of the 180 species of *Linum* genus that belongs to the *Linnaceae* family (2). Flax seeds being part of traditional nutrition are a part of the human diet in many countries for many years (3). Flax seeds are being vastly cultivated in China, India, USA, Pakistan, and Australia and have been investigated for numerous uses including their medicinal effects (4). These plants are commercially used as a large source of linseed oil, fiber, and ornamental purposes (5). In recent years, studies have proved flax seeds as a health-potentiating medicinal and functional food due to the presence of a remarkable amount of bioactive compounds (6).

Major bioactive constituents of flax seeds having a significant effect on the body are  $\alpha$ -linolenic acid, soluble mucilage, saturated and unsaturated fatty acids, and various minerals (calcium, phosphorus, and magnesium). The nutrient content of brown and yellow flax seeds has been proven almost the same by different researchers (7). The existence of mentioned bioactive compounds and minerals in flax seeds increases their medicinal importance. It has been used in traditional medicine for years and abundant literature is present explaining the health-promoting effects of flax seeds (4). Multiple studies demonstrate the antioxidant (8), anti-inflammatory (9), analgesic (10), antidiabetic (10), antihyperglycemic (11), anticancer (12), antifungal (13) and antibacterial (14) activity of flax seeds that proves it as a significant plant source which needs a lot more investigation in the medicinal field. Regarding the antibacterial activity, in particular, results of an in vitro study showed a promising inhibitory activity by ethanolic extract of flax seeds against *B. cerues*, *S. aureus*, *Ps. Aeruginosa* and *K. pneumoniae* (15). Another research proved the bactericidal activity of flax seeds against *E. coli*, *P. vulgaris*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* (16).

The main causative organism responsible for dental caries is *Streptococcus mutans*. Unfortunately, there is a lack of enough literature reporting the antibacterial activity of flax seeds against the bacteria present in the oral cavity, causing dental caries and periodontal diseases. But still few studies have reported bactericidal activity of flax seeds against prominent disease-causing bacteria of the oral cavity including *Porphyromonas gingivalis* (17), *Staphylococcus* (18), and *Lactobacillus*. Inhibitory or bactericidal activity of *Streptococcus mutans*, being the main cause of dental caries, should be investigated with herbs like flax seeds which have bactericidal effects on a vast majority of bacteria. Achieving better results against *Streptococcus mutans* colonies will allow us to use flax seeds extract in different mouth rinses or toothpastes. So, the bactericidal activity of flax seeds will reduce the incidence of highly prevalent dental caries disease

Comment [L12]: ???

Comment [L13]: delete

Comment [L14]: *P. aeruginosa*

Comment [L15]: delete

Comment [L16]: insert period

Comment [L17]: delete

which, in the future, can progress to other severe periodontal diseases.

**Comment [L18]:** check formatting of all work, including fonts, spelling and grammar

## Methodology:

**Comment [L19]:** delete

It was a preclinical experimental study conducted at Baqai Medical and Dental College Karachi, from December 2021 to January 2022. Calculated sample size was  $n = 60$ . Consecutive sampling technique was used to recruit the participants. The participants were given an envelope for group randomization. Film of plaque from labial surface of teeth of study participants was collected on sterile strips that was transported to laboratory for culture in sterile containers. For culture *S. mutant* samples were inoculated in Columbia Agar with 5% sheep blood and incubated for 48 h at 37 °C and increased level of CO<sub>2</sub>. Flax seeds (1000-gram) were purchased from local market of Karachi and authentication number i.e. Specimen voucher 911 was allotted. The seed were washed and shed dried and lastly was grinded to powder form. The seeds were soaked in 2500 ml of 90% ethanol for 15 days with intermittent. After 15 days the filtrate was filtered with Whatman filter paper (number 1) that was further processed at 60°C by using water bath. The mixture was than dried at 50°C until a well concentrated extract was produced on rotary evaporator. The extract was kept in an airtight bottle and stored in a refrigerator till usage. The extract was diluted in distilled water in 1:4 (Extract: Distilled water) concentration. Study participants were instructed to not brush their teeth before sampling. Study participants were divided into three groups (negative control, positive control, flax seed extract group) each group had 20 participants. Diluted flax seed extract was given to experimental group for rinses, distilled water was given to negative control group and Positive controls were given a commercially available mouth rinse. Next sample of plaque was collected after two hours to observe the effects of flax seed extract on bacterial colonies. ANOVA followed by post hoc Tukey was applied to identify the inter and intra group comparison and Paired t test was applied as test of significance for pre and post experimental comparison, <0.05 p-value was considered as significant at 95% confidence interval.

**Comment [L20]:** idem L15

**Comment [L21]:** CO<sub>2</sub>

**Comment [L22]:** mL

**Comment [L23]:** idem L9

**Comment [L24]:** idem L7

**Comment [L25]:** %

## Results:

Out of sixty participants 27 (45%) were males and 33 (55%) were females the mean age of participants was  $33 \pm 4.8$ . when the participants were asked about brushing habit 49 (81.6%) responded that they brush their teeth daily. Figure 1 shows the demographic data of study participants. The growth of colonies was calculated on growth media plates in samples prior to rinsing and samples that were taken after the rinsing. The pre and post samples showed significant (p-value <0.05) decrease in number of colonies in positive control group (conventional rinse) and flax seed extract group as shown in table 1. The intra group comparison of negative and positive control showed significant difference in number of colonies and same was observed with the flax seed extract rinse. However, the positive control and flax seed extract comparison was insignificant. Table 2 shows the intra group comparison of experiment.

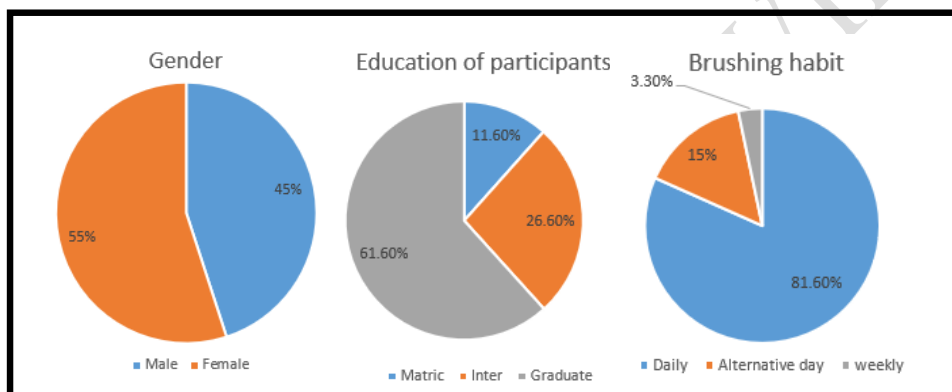


Figure 1. Demographic representation of participants

Table 1. Number of colonies before and after rinses in negative control, positive control and experimental groups

	Negative Control	Positive control	Flax seed extract
<b>Before</b>	$9 \times 10^4$	$9 \times 10^4$	$10 \times 10^4$
<b>After</b>	$10 \times 10^4$	$5 \times 10^4$	$4 \times 10^4$
<b>P value</b>	0.417	0.001*	0.002*

Table 2: Post hoc analysis among groups

Groups wise comparison				p- value
Negative control	$10 \times 10^4$	Positive control	$5 \times 10^4$	0.005*
Negative control	$10 \times 10^4$	Flax seed extract	$4 \times 10^4$	0.010*
Positive control	$5 \times 10^4$	Flax seed extract	$4 \times 10^4$	0.092

## Discussion:

Comment [L26]: delete

Comment [L27]: Idem L7

Comment [L28]: cite at the foot of the table

Comment [L29]: ???

Comment [L30]: Idem L25

Comment [L31]: remove underlining and colon

Even after vast advancement in medical sciences, the oral infections are still a burden on human life. Beside this, newer investigations on various natural herbs are also being carried out to study the hidden beneficial effects of natural herbs. Considering various medicinal properties of flax seeds or *Linum usitatissimum* mentioned above, it was also important to observe its bactericidal effects against the most prominent bacteria of oral cavity, that is, *Streptococcus mutans*. Contribution of *S. mutans* in the process of dental caries initiation and progression makes it an important aspect to research about it along with plants having bactericidal properties like flax seeds.

The overall results of our study showed a significant antibacterial activity of flax seeds. 100gm of *Linum usitatissimum* was used and its ethanolic extract was obtained which was further diluted with distilled water. Another study used the same extraction method but the used the aqueous and alcoholic extracts to monitor the antibacterial activity of flax seeds (14). In our study the total 60 participants, including males (45%) and females (55%) both, were divided into 3 groups having 20 participants in each group. Locally available mouth rinse was given to the group 1, that is positive control group. Distilled water was given to the group 2, that is negative control group. The third, experimental group, received diluted flax seeds extract. No significant increase in number of bacterial colonies was reported in negative control group where rinse with done only with the distilled water. While the use of positive control and flax seeds extract rinses decreased the number of bacterial colonies significantly. Group wise comparison showed significant inhibitory effect on bacterial growth while comparing the flax seeds extract group and negative control group. Almost same inhibitory effect was observed while comparing the negative control group and positive control group.

There is a lack of enough literature about antibacterial activity of flax seeds against periodontal bacteria. An *in vitro* study reported that the flax seeds extract is efficacious against *P. gingivalis* which substantiated its bactericidal potency (17). Another study reported inhibitory effect on the *S. aureus* growth and its proliferation cycle by the nano-emulsion of *L. usitatissimum* seeds essential oil. This study also reported the antioxidant effect of flax seeds by scavenging the free radicals (18). On the other hand, a study was done to analyze the bactericidal activity of flax seeds different extracts by inhibiting the growth of four multi-resistant clinical bacterial isolates that are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The results showed that the crude and aqueous extracts of *Linum usitatissimum* did not show any inhibitory effects on all the four above mentioned strains at different concentrations. Contrarily,

**Comment [L32]:** First time appearing in the text, write full name: *Porphyromonas gingivalis*

the methanolic extract of *Linum usitatissimum* oppositely enhanced the growth of all of the bacterial strains at the concentration of 0.15mg/mL or above (19). This contradiction emphasizes that more *in vivo* and *in vitro* studies should be done to further validate the antibacterial activity of flax seeds extract.

Comment [L33]: mL

A study done in 2018, demonstrated the various biologically active constituents of flax seeds possessing antibacterial activity by GC-MS screening. The same study also evaluated the antibacterial activities against *S. mutans*, *S. pyogenes*, *E. faecalis*, and *L. casei* as compared to n-hexane and dichloromethane extracts. This study reported significant bactericidal activity against all these bacteria and emphasized on further investigations (20). Another study done in International Islamic University Malaysia investigated the bactericidal effects of methanolic extract of *Linum usitatissimum* against *Streptococcus pyogenes*, *Streptococcus mutans*, *Lactobacillus casei*, *Enterococcus faecalis* and *Candida albicans* using disc diffusion and broth dilution methods. This study also aimed to investigate the mode of action of flax seeds on the bacterial cell membrane. The results reported remarkable antibacterial activity by flax seeds extract and its action on the cell membrane of mentioned oral pathogens (21).

Thus, the above-mentioned studies including our research, supports the argument that the bactericidal activity of naturally produced flax seeds extract is almost equal to the antibacterial activity of synthetically made mouth washes against various oral pathogens along with other beneficial effects. So there is a need for interventional approach through further *in vivo* and *in vitro* studies that may assess the favorable effects of flaxseed extract in people to prevent dental caries or in patient with chronic periodontal diseases.

#### Conclusion:

Flax seeds extract mouth rinse reduced the number of colonies equally as that of commercially available mouth rinse. Exploration of flax seeds extract rinse and its phytoconstituents against *Streptococcus mutans* is recommended to validate its role as potential herbal antibacterial mouth rinse.

**ERC:** The study was approved by ethics review board of university.

#### References:

1. Ahmad M, Khattak MR, Jadoon SA, Rab A, Basit A, Ullah I, et al. Influence of zinc sulphate on flowering and seed production of flax (*Linum usitatissimum* L.): A medicinal flowering plant. 2019;14(4):464-76.
2. Ansari R, Zarshenas MM, Dadbakhsh AHJCddt. A Review on Pharmacological and Clinical Aspects of *Linum usitatissimum* L. 2019;16(2):148-58.
3. De LJIRSR. Edible seeds and nuts in human diet for immunity development. 2020;6(11):38877-81.
4. Khare B, Sangwan V, Rani VJJoFP, Preservation. Influence of sprouting on proximate composition, dietary fiber, nutrient availability, antinutrient, and antioxidant activity of flaxseed varieties. 2021;45(4):e15344.
5. Kluza-Wieloch M, Waśkiewicz A, Bednorz L, Nowińska RJJöE. The content of selected elements in common flax seeds (*Linum usitatissimum* L.) depending on the cultivar and weather conditions. 2020;25(3).
6. Garros L, Drouet S, Corbin C, Decourtil C, Fidel T, Lebas de Lacour J, et al. Insight into the influence of cultivar type, cultivation year, and site on the lignans and related phenolic profiles, and the health-promoting antioxidant potential of flax (*Linum usitatissimum* L.) seeds. 2018;23(10):2636.
7. Raghuwanshi V, Agrawal R, Mane KJJPP. Flaxseed as a functional food: a review. 2019;8:352-4.
8. Safdar B, Pang Z, Liu X, Jatoi MA, Mehmood A, Rashid MT, et al. Flaxseed gum: Extraction, bioactive composition, structural characterization, and its potential antioxidant activity. 2019;43(11):e13014.
9. Asad B, Khan T, Gul FZ, Ullah MA, Drouet S, Mikac S, et al. Scarlet Flax *Linum grandiflorum* (L.) In Vitro Cultures as a New Source of Antioxidant and Anti-Inflammatory Lignans. 2021;26(15):4511.
10. Mechchate H, Es-Safi I, Conte R, Hano C, Amaghnouje A, Jawhari FZ, et al. In Vivo and In Vitro Antidiabetic and Anti-Inflammatory Properties of Flax (*Linum usitatissimum* L.) Seed Polyphenols. 2021;13(8):2759.
11. Qureshi JA, Memon Z, Ismail K, Agha S, Saher F, Motiani V. ANTI HYPERGLYCEMIC AND ANTI DYSLIPIDEMIC EFFECTS OF FLAX SEEDS (*LINUM USITATISSIMUM*) EXTRACT IN DIABETIC RATS MODEL.
12. Tannous S, Haykal T, Dhaini J, Hodroj MH, Rizk SJB, Pharmacotherapy. The anti-cancer effect of flaxseed lignan derivatives on different acute myeloid leukemia cancer cells. 2020;132:110884.
13. Madheslu M, Sadasivam N, Ameerbasha SS, Ramakrishnan V. Toxicity of Tungsten Oxide and IAA-Loaded Tungsten Oxide Nanoparticles on *Linum usitatissimum* Germination and Their Antifungal Activity. Cellular and Molecular Phytotoxicity of Heavy Metals: Springer; 2020. p. 403-18.
14. Hussien ZG, Aziz RAJSRiP. Chemical Composition And Antibacterial Activity Of *Linum Usitatissimum* L.(Flaxseed). 2021;12(2):145-7.
15. Dagnaw M. Isolation of antibacterial compounds from *Linum usitatissimum* and evaluation its antibacterial activity. 2019.
16. Borkar N, Murarkar K. ANTIMICROBIAL ACTIVITY OF FLAXSEED (*L. USITATISSIMUM*) OIL AND LIMESTONE WATER AGAINST PATHOGENIC MICROORGANISMS. 2018.

17. Badiger AB, Gowda TM, Rajarajeshwari S, Saswat S, Majhi TK, MEHTA DJIJoHM. Antimicrobial effect of flaxseed (*Linum usitatissimum*) on periodontal pathogens: an in vitro study. 2019;7(3):16-9.
18. Keykhasalar R, Tabrizi MH, Ardalan PJJJI. Antioxidant property and bactericidal activity of *linum usitatissimum* seed essential oil nanoemulsion (LSEO-NE) on *Staphylococcus aureus*. 2020;7(2):e101639.
19. Na'was T, Abou Raji El Feghali P, Ibrahim R. Antibacterial activity of *curcuma longa*, *opuntia ficusindica* and *linum usitatissimum*. 2018.
20. Fadzir UA, Mokhtar KI, Mustafa BE, Darnis DSJMJoHR. Evaluation of bioactive compounds on different extracts of *linum usitatissimum* and its antimicrobial properties against selected oral pathogens. 2018.
21. Fadzir UA. Antimicrobial activities of *Linum usitatissimum* extracts on selected oral pathogens: an in vitro study: Kuantan, Pahang: International Islamic University Malaysia, 2018; 2018.