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ANTIMICROBIAL ACTIVITY OF FLAXSEED (*L.USITATISSIMUM*) OIL AND LIMESTONE WATER AGAINST PATHOGENIC MICROORGANISMS

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ABSTRACT

Traditional medicines make use of natural products and are of great importance. The aim of present study is to investigate in vitro antimicrobial activity of flaxseed oil, flaxseed, and flaxseed powder and limestone water with various formulations against pathogenic *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus vulgaris.* From the literature survey it is found that flaxseed oil, flaxseed and limestone can be used on wounds, burning wounds, cuts. The experimental result showed that the mixture of flaxseed oil and limestone water remarkable have antimicrobial activity against pathogenic microorganisms except *Proteus vulgaris,* and therefore have great potential to use in pharmaceutical formulations of ointments.

KEYWORDS: Antimicrobial activity, flaxseed oil, limestone water, test pathogenic microorganisms

INTRODUCTION

Traditional medicines make use of natural products, and are of great importance. Such forms of medicine as traditional Chinese medicine, Ayurveda, Kampo, traditional Korean medicine and Unani employ natural products and have been practiced all over the world for hundreds or even thousands of years, and they have blossomed into orderly-regulated system of medicine(Yuan H. 2016). Plants have long provided mankind with a source of medicinal agents, with natural products serving as the source of all drugs (Balandrin *et al.*

1993).Medicinal plants form the backbone of traditional medicine, have in the last few decades been the subject of very intense pharmacological studies.

Flaxseed (*L.usitatissimum*) and flaxseed oil have been valued as foods and for medicinal purposes since ancient times(Vaisey-Genser and Morris 2003). Flaxseed is now attracting increased interest as a food due to its high fibre content, high content of bioactive phenolic compounds(mainly lignans) and high content of the essential omega -3 fatty acid alphalinolenic acid(ALA)(Dean 2003). Flaxseed lignin such as secoisolariciresinols(SDG), the mammalian lignans such as enterodiol and enterolactone acts as antioxidant(Kitts D.D. *et al* 1999). Its growing popularity is due to health imparting benefits in reducing cardiovascular diseases, decreased risk of cancer, particularly of the mammary and prostate gland, anti-inflammatory activity, laxative effect, and alleviation of menopausal symptoms and osteoporosis.

Flaxseed raw oil is used as an astringent in fungicidal lotion and as an insecticide and has moderate insect repellent properties(The wealth of India 2006). In earlier studies the *L.usitatissimum* fixed oil has been reported to exhibit significant anti-inflammatory(Kaithwas G. et al 2011) antiarthritic(Kaithwas G. and Majumdar D.K.2010), antiulcer(Kaithwas G. and Majumdar D.K.2010), and antidiebetic (Kaithwas G. and Majumdar D.K.2012) properties. The antimicrobial activity of *L.usitatissimum* oil and its therapeutic efficacy in bovine mastitis, an inflammatory disorder caused by microbial infection has been reported recently(Kaithwas G. *et al*., 2011).

The Limestone is anti-biotic, anti-pyretic, anti-fungal and anti-inflammatory. Regular-controlled use of limestone is effective in fighting hepatitis (all types, i.e. A, B, C, D, E), Jaundice primary infertility, height, breast development, eye vision, osteoporosis, musculoskeletal pains – arthritis – spinal cord related problems – sciatica pain, hair fall problems menopause, teeth sensitivity(http://www.theayurveda.org/ayurveda/herbal-medicine/5-natural-treatment-can-using-limestone/). It is reported apart from healing pain from internal injuries, limestone is also excellent at treating and curing external cuts and wounds if honey and limestone thick paste applied on the cut. It will not only stop the bleeding, but also help in healing the cut faster(https://i0.wp.com/deveev.com/wp-content/uploads/2016/09/It-can-heal-wounds.jpg?w=650). It is reported that limestone powder and honey paste cure all the pain anywhere in the body i.e. hands and legs swells due to the injury.

In the present work we studied the antimicrobial activity of flaxseed, flaxseed oil and limestone water individually and in a mixture form.

METHODS AND MATERIAL

Test pathogens

Pure culture of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris* were used in this study. It was subcultured on nutrient agar slant for 24 hours before use.

Collection of flaxseed and limestone

Flaxseed, flaxseed oil and limestone were purchased from local market.

Preparation of limestone water

Limestone was dissolved in water for 3 days; water was separated to remove all the unwanted impurities from it. Then limestone was again dissolved in water and mixed well. This mixture was stand for 15 minutes to settle down the limestone at the bottom. The limestone water floats at upper surface was separated, and stored in a glass container for further use.

Procedure for preparation of different extracts

(A) Hundred ml (100ml) flaxseed oil and 25 ml limestone water (4:1 v/v) was mixed for few minutes to get yellow colour. (B) Hundred grams (100 grams) of flaxseed was soaked in 50 ml distilled water (2:1 w/v) for making of paste.(C) Pretreated limestone water.(D) Two grams flaxseed and 2 ml distilled water was mixed for making powder extract (1:1w/v).(E) Two grams flaxseed paste and 1ml distilled water (2:1w/v).(F) Flaxseed oil.

All the extracts were stored in air tight glass containers for testing antimicrobial activity.

Antimicrobial activity

Antimicrobial activity was tested by Kirby-Bouer method using Muller Hingtone agar. Twenty four hours old one loopful culture was inoculated in 25 ml sterilized nutrient broth, and incubated overnight. This 0.1 ml test broth culture was used for spread plate method. The well size for this experiment was 2 mm. The volume of extract was 0.1 ml in one well. Control was also set for this experiment. Plates were kept in the incubator at 37°C for 24 hrs for the observation of zone of inhibition. All the extracts (A-F) were tested against each organism following the same volume of extract and test culture of organisms.

EXPERIMENTAL RESULTS

(a) *E. coli* **(b) *S. aureus*** **(c) *K. pneumoniae*** **(d) *Ps. aeruginosa***

Photograph 1: Antimicrobial activity of flaxseed oil and limestone water mixed extract against test pathogenic microorganisms.

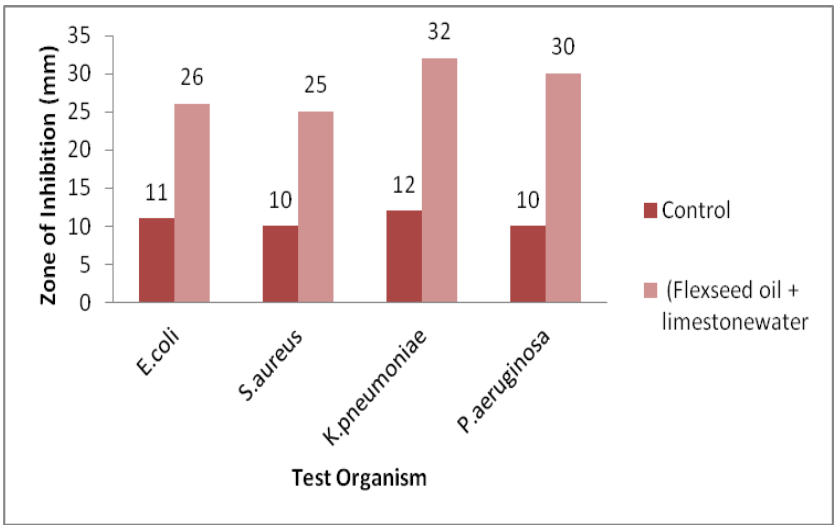


Figure 1: Antimicrobial activity of flaxseed oil and limestone water (A) against test pathogenic microorganisms.

(a)*E. coli* (b) *S. aureus* (c) *K. pneumoniae* (d) *Ps. aeruginosa* (e) *P. vulgaris*

Photograph 2: Antimicrobial activity of flaxseed paste (B) against test pathogenic microorganisms.

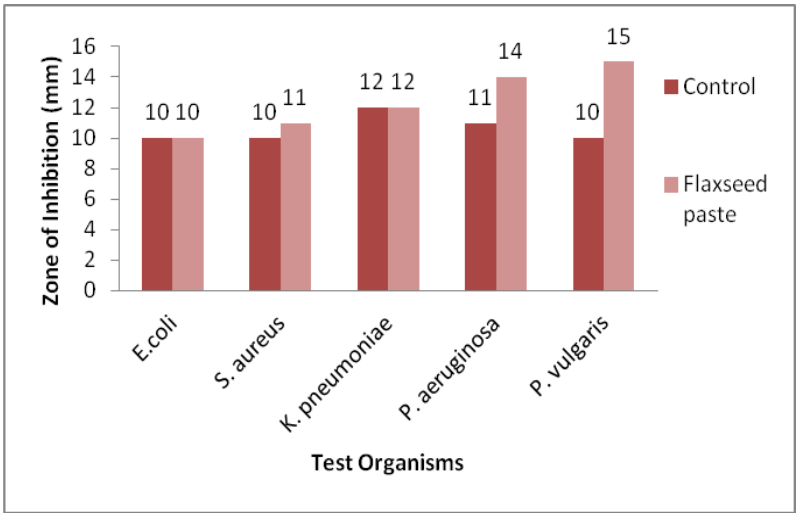
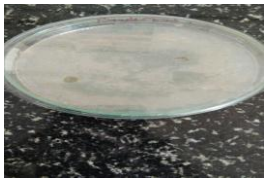


Figure 2: Antimicrobial activity of flaxseed paste (B) against test pathogenic microorganisms.



(a) *E. coli*

Photograph 4.Antimicrobial activity of flaxseed powder and distilled water (D) against test pathogenic microorganisms.

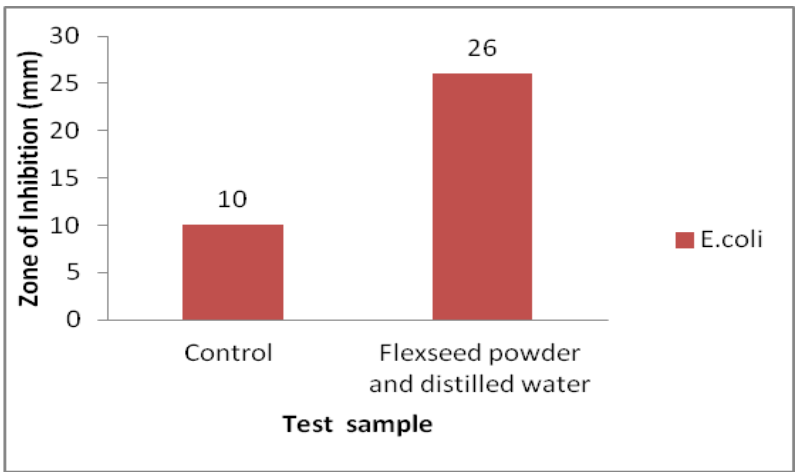
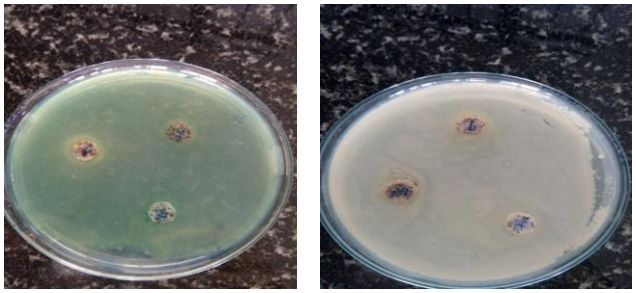


Figure 4: Antimicrobial activity of flaxseed powder and distilled water (D) against test pathogenic microorganisms.



(a)*Ps. aeruginosa* *(b) P. vulgaris*

Photograph 5: Antimicrobial activity of flaxseed paste and distilled water (E) against test pathogenic microorganisms.

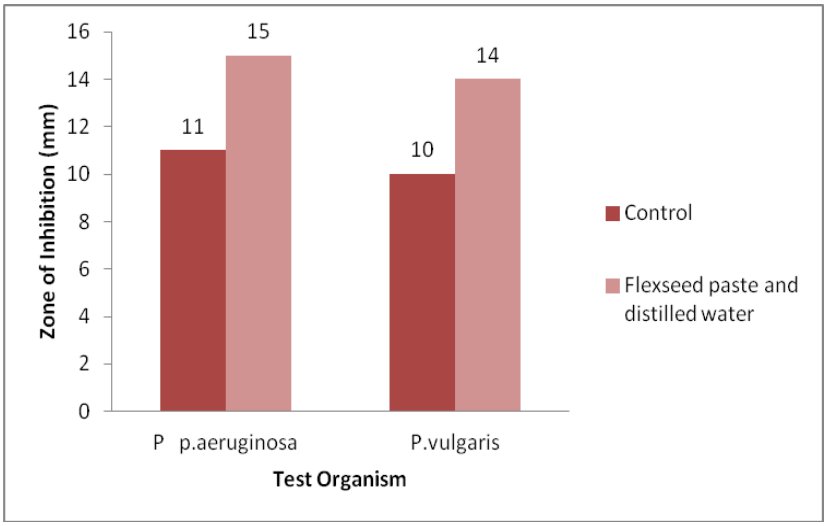


Figure 5: Antimicrobial activity of flaxseed paste and distilled water (E) against test pathogenic microorganisms.

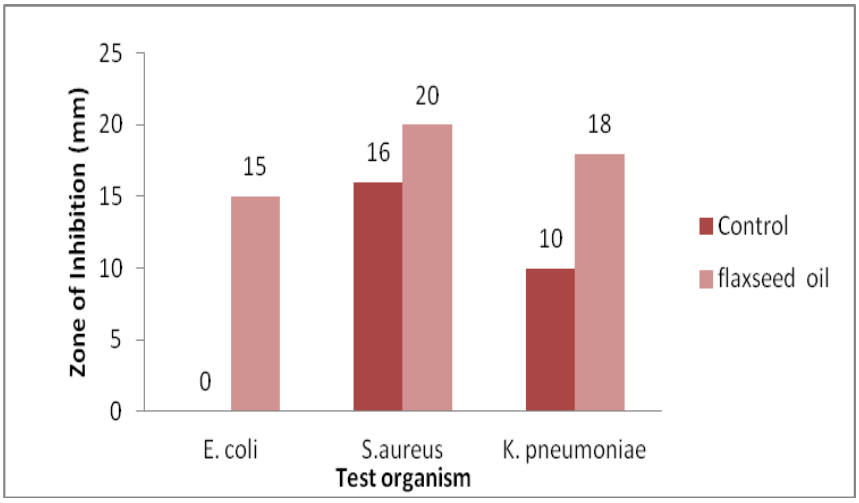


Figure 6: Antimicrobial activity of flaxseed oil (F) against test pathogenic microorganisms.

DISCUSSION

The antibacterial activity of test organism was observed in the form of zone of inhibition measured in millimeter (mm) is shown in figures. Depending upon the active ingredient and diffusion capacity into agar media, flaxseed oil and limestone water mixture (figure 1) had shown the highest antimicrobial activity against the test microorganism except for *proteus vulgaris.*

The extracts of flaxseed powder and water had shown the antimicrobial activity only against *Escherichia coli(*figure 4). The extracts of flaxseed paste (figure 2) and flaxseed paste with distilled water (figure 5) had shown very low antimicrobial activity against *Pseudomonas aeruginosa* and *Proteus vulgaris*. Flaxseed oil was very effective against *E.coli* than *Pseudomonas aeruginosa* and *S.aureus* respectively. The limestone water extract had not shown any antimicrobial activity against any test microorganisms.

Joshi Y. (2014) has been reported that linseed oil synergies the antimicrobial potential of gemifloxacin when used simultaneously in various combinations. Alaa A. Gaafar (2013) published a paper on i*n vitro* antioxidant and antimicrobial activities of lignan flax seed extract against *Bacillus subtilis* NRRL B-94, *Escherichia coli* NRRL B-3703, *Psedumonas aeruginosa* NRRL, *Staphylococcus aureus* NRRL, *Aspergillus niger* NRRL313, and *Candida albicans* NRRL 477, and the results were considered as most important and promising finding in pharmaceutical properties of lignans. Firas A. Al-Bayati (2007) was studies on antibacterial activity of *Linum usitatissimum* L. seeds and active compound detection against the *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using agar-well diffusion method, and compared their antibacterial activities with the antibiotics Ampicillin, Cefalexin, Chloramphenicol and Tetracycline. Flaxseed oil is highly valued in folk medicine. It is very effective when applied externally, on the skin. It is used for healing scars, burns, inflammation, eczema, psoriasis; skin rash (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2935806/).It is mentioned in Ayurveda flaxseed oil also helps in speeding up the healing of skin lesion. (http://home of ayurveda.org/ flex-seeds-in ayurveda/and ht=en –gif).It is reported limestone apart from healing pain from internal injuries it is also excellent at treating and curing external cuts and wounds if honey and limestone thick paste applied on the cut. It will not only stop the bleeding, but also help in healing the cut faster (https://i0.wp.com/deveev.com/wp-content/uploads/2016/09/It-can-heal-wounds.jpg?w=650).

CONCLUSION

The present study suggested that mixture of flaxseed oil and limestone water have a great potential as antimicrobial agents against selected enteric pathogenic microorganisms, and they can be used as an alternative medicines in the treatment or control of enteric bacterial infection.The results of antimicrobial activity of this mixture reveals the presence of medicinally important constituent in this mixed extract. Many evidences gathered in earlier studies which confirmed the bioactivity of flaxseed oil and limestone water. Therefore mixture of flaxseed oil and the limestone water extract could be seen as a good source for useful drug, and could be helpful for wounds, burning wounds and cuts skin pathogens.

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