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PRELIMINARY SCREENING FOR ANTIBACTERIAL POTENTIAL OF METHANOL EXTRACT OF FLAXSEED (*LINUM USITATISSIMUM*)

Research Article

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ABSTRACT

Flax (*Linum usitatissimum*) is one of the oldest cultivated plants, which are utilized for oil, fiber, food and some medical purposes. The antibacterial properties of flaxseed are poorly studied. Methanolic crude extract of the seeds of flax (flaxseed) was screened for the antibacterial activity against 4 different bacterial strains, namely *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027, using the disc-diffusion method. The results exhibited weak antibacterial activity, the inhibition zones were ranging between 7.5±0.5 to 7.0±0.0 mm. Accordingly, further studies are recommending using different extraction methods, in order to evaluate the potential antibacterial properties of this plant product.

Keywords:*Linumusitatissimum*, disc diffusion test, crude extract, Gram-positive, Gram-negative bacteria

INTRODUCTION

Screening and exploration of bioactive natural products has been widely implemented in the 18th and 19th centuries which led to tremendous pharmaceutical discoveries and isolation of pure curative compounds, most of these compounds came from plant origin and from ethno botanical information, among these discovered "natural" drugs are digoxin from foxglove (digitalis), morphine from poppies, aspirin from salicylic acid in willow bark and penicillin from mold **(Rishton, 2008).** The following two centuries (20th and 21st) witnessed a remarkable transformation from natural drugs to synthesized medications, but surprisingly interest in natural products have revived again in the 21st century after the growing scientific studies and reports suggested that synthetic drugs could have a serious side effects and other complicated problems on the human health (**Rates, 2001).** Since 1997, the scientific communities and international health organizations alarming from the growing phenomenon of antibiotic-resistant pathogens which will become catastrophic soon, and called to make further efforts in order to discover new antimicrobial agents, urgently **(Wise, 2011).** Accordingly, plants of ethno pharmacological background could be a good source for new antimicrobial drugs.

Flaxseeds (*Linum usitatissimum* L.), also known as flax or Linseeds, the plant is an annual herb belonging to family Lineaceae, Almost all parts of this plant are consumed or used for fiber, oil, nutritional products and some medicinal purposes **(Kajla *et al.*, 2015).** Flaxseeds contain approximately 30% dietary fibers, claimed to be able to control hyperglycemia and hypercholesterolemia in humans, it contains also 40% oil, rich in Omega-3 and Alpha-Linolenic acid. Omega-3 is well known of its benefits to promote health, while alpha-Linolenic acid could prevent cancers and heart diseases; In addition to good quantities of proteins and phenolic compounds such as cinnamic acids, coumarins, flavonoids and lignans **(Xu, 2007).** The antibacterial potential of flaxseed is poorly studied. The aim of the current study is to evaluate the potential antibacterial activity of flaxseeds (*Linum usitatissimum*).

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MATERIALS AND METHODS

Plant material

Dry seeds of flax (*Linum usitatissimum*) were purchased from a local herbal market from Qassim (Figure 1), identified, cleaned from any impurities and washed with distilled water.

Figure 1: The flaxseeds *Linum usitatissimum*

Bacterial strains

Four referenced bacterial strains were used in this investigation, representing Gram-positive and Gram-negative bacteria, which were *Staphylococcus aureus* ATCC® 29213TM, *Bacillus cereus* ATCC® 10876™ (Gram-positive bacteria) and *Escherichia coli* ATCC® 25922™, *Pseudomonas aeruginosa* ATCC® 9027™ (Gram-negative bacteria).

Plant extraction

Flaxseeds (50 grams) were macerated in 500 mL methanol (70%) in a well tighten dark container which put on a shaker with a magnetic stirrer (at 200 r.p.m.) and left for up to two days at room temperature. Then, filtered using a muslin cloth and residues was discarded. The filtrate was filtered again using Whatman No.1; the filtrate was evaporated under reduced pressure using a Rotary evaporator to get concentrated solution, which was transferred to the autoclave in a wide glass dish at 40 °C for up to two days to get rid of the water residues. The dry extract yielded was approximately 3.2 grams.

Preparation of inoculums

Referenced bacterial strains were cultured in nutrient broth for up to 18 hours at 37°C to reach the exponential growth phase. Then, adjusted to 0.5 McFarland's standard, using a sterile normal saline solution, to get bacterial density equivalent to about 1.0×10^8 CFU/ml (Colony forming unit per ml), which were used the disc-diffusion test.

Disc diffusion test

The antibacterial activity of methanol extract of flaxseeds was evaluated using a modified Kirby-Bauer disc diffusion method as reported by **Abdallah (2016)** with some modifications; The dried methanol extract was reconstituted in the same solvent (methanol 70%) to get a working concentration 100 mg/mL, The pre-experimental test showed that methanol has no inhibitory effect on bacterial growth. Also, sterile plates (90 cm in diameter) containing an autoclaved nutrient agar were prepared. These plates were inoculated with the adjusted bacterial strains using a sterile cotton swab. A sterile blank disc (previously prepared from Whatman No.1 filter paper) were saturated with 100 mg/mL (6 mm Whatman No.1 paper disc absorb about 15-20 µL, based on the nature of the crude solution) and placed onto the inoculated plate. A disc saturated with 5-mg/mLchloramphenicol (positive control) and discs saturated with 70% methanol (negative control) were also loaded on the seeded plates. Then, the plates were incubated for 24 hours at 37°C. After that, the susceptibility of tested bacteria was determined by measuring the clear zone of growth inhibition in millimeter (mm) using a transparent ruler. The test was repeated twice and means ± standard errors were calculated.

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RESULT AND DISCUSSION

The results of antibacterial activity tests are presented in Table 1, Figure 2 and 3. In general, the methanolic extract of flaxseed (100mg/mL) exhibited weak antibacterial activity compared with the chloramphenicol (5 mg/mL). The mean zone of inhibition of the Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 10876) was 7.5±0.5 mm, compared with the chloramphenicol which was ranging between 33.5±1.5 and 34.0±1.0 mm. Whereas, the mean zone of inhibition of the Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027) was 7.0±0.0, compared with the chloramphenicol, which was ranging between 13.0±1.0 and 28.5±0.5 mm. Reports on the antibacterial activity of flaxseed are scant. A study on the oil of flaxseed showed no antibacterial activity on *Escherichia coli* or *Enterococcus faecalis*, but showed antibiofilm activity against some bacterial strains **(Al-Mathkhury *et al.*, 2016).** The current study is in contradiction with **Al-Bayati (2007)** who mentioned that the petroleum ether extract showed significant antibacterial activity against four different Gram-negative bacteria, while the chloroform extract recorded weak activity. It is also noted that, there is a contradiction between the results of different studies on the same extraction solvent, **Amin and Thakur (2014)** published that the Chloroform extract of flaxseed was the most effective extract against different bacterial strains, while **Al-Bayati (2007)** claimed that the chloroform extract showed weak antibacterial activity. Which require more advanced studies in order to clarify these findings. However, various plant varieties, source of the plant, environmental conditions and different extraction methods may influence on the findings and could gives different antibacterial results from the same plant depending on the concentrations and nature of the bioactive compounds. Accordingly, it is important to conduct additional investigations on flaxseed using different extraction methods, fractionation and isolation of is compounds. Finally, an interesting study conducted by **Tehrani *et al.*, (2014)** on the flaxseed proteins and carbohydrates found that, the flaxseed proteins inhibited the growth of the tested bacteria, while the flaxseed carbohydrates might cover the inhibitory effect of flaxseed proteins and promote the bacterial growth.

Table 1: The antibacterial activity of the methanolic extract of flaxseed

Tested compound	Mean zone of inhibition ± standard error of means (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	Sa	Bc	Ps	Ec
Methanol extract (100mg/mL)	7.5±0.5	7.5±0.5	7.0±0.0	7.0±0.0
Chloramphenico (5 mg/mL)	33.5±1.5	34.0±1.0	13.0±1.0	28.5±0.5

*Mean of two replicates, Sa=*Staphylococcus aureus* ATCC 29213, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 25922, Ps=*Pseudomonas aeruginosa* ATCC 9027.

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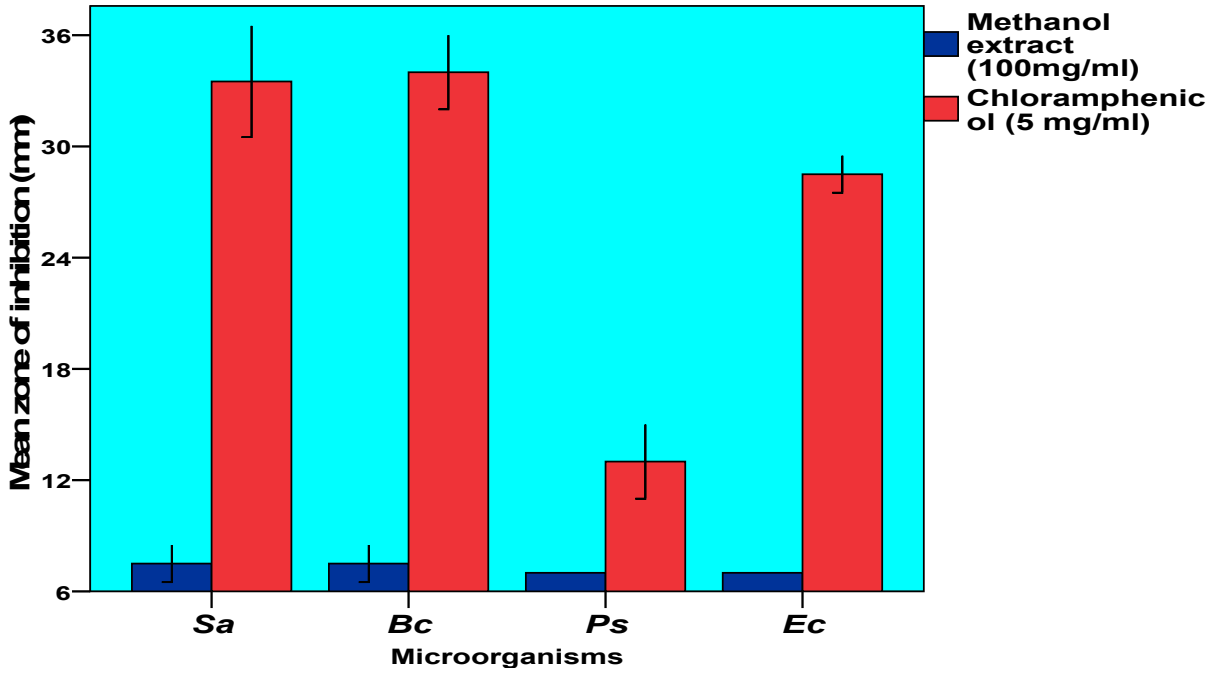

Figure 2: The antibacterial efficacy of methanolic extract of flaxseed compared with the antibiotic*

*Mean of two replicates, diameter of the paper disc (6mm) was omitted in the graph, Sa=*Staphylococcus aureus* ATCC 29213, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 25922, Ps=*Pseudomonas aeruginosa* ATCC 9027.

Figure 3. Representative photo of disk diffusion test showing weak zone of inhibition

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CONCLUTION

Flaxseed (*Linum usitatissimum* L.) is used since antiquity, for oil, food and treatment from some disorders and ailments. It is also considered as a rich source of nutritive and bioactive compounds. However, little is known about its antibacterial activity. The current study revealed weak antibacterial activity of the methanol extract (70%). Although, some reports mentioned presence of phenolics and flavonoids in flaxseed. Accordingly, this study suggests using successive extraction with different solvents, which could reveal the presence of possible antibacterial compounds, if any. Moreover, these phytochemical compounds may have other bioactive effects on human body rather than the antibacterial activity since it is rich in oil and some important fatty acids, which require further future integrative investigations.

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