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Antibacterial Activity and Phytochemical Screening of Linum Usitatissimum L. on Bacteria Isolated from Wound Infections

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ABSTRACT

Aims Wound infections are one of the most important problems in human life. Continued use of antimicrobial agents against wound infections leads to the appearance of antibiotic-resistant bacteria strains. Flaxseed has important pharmacological properties against various diseases. This study aimed to determine the antibacterial activity of flaxseed extracts against different bacteria isolated from wound infections.

Materials & Methods This experimental study used phytochemical screening for flaxseed extracts. Also, the agar well diffusion method was used to examine the antibacterial activity of aqueous, ethanolic extract, and flaxseed oil (200, 100, 50, and 25mg/ml) against gram-positive and gram-negative bacteria.

Findings Phytochemical screening for the crude aqueous and ethyl extracts of flaxseeds revealed alkaloids, terpenoids, tannins, flavonoids, glycosides, saponins, steroids, phenolic compounds proteins, and carbohydrates. Aqueous extract showed inhibition zones only for Staphylococcus aureus and Streptococcus faecalis at 50, 100, and 200mg/ml. All the concentrations of the ethanolic extract showed inhibitory effects against all tested bacteria. The maximum antibacterial effect of ethanol extract on aqueous extract was 200mg/ml. Also, 200mg/ml of flaxseed oil had the most powerful inhibitory effects on all tested bacteria

Conclusion Flaxseed ethanolic extract and flaxseed oil have an inhibitory effect against different species of both gram-positive and gram-negative bacteria isolated from wound infections.

Keywords Linum usitatisimum; Antibacterial Activity; Flaxseed Extract; Male Phytochemical Screening; Wound Infection

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Introduction

Wound infection remains a difficult condition that imposes a significant healthcare burden. Early detection and successful treatments are more important for limiting its economic and health effects and antibiotic resistance [1]. Infection in a wound is a major barrier to healing and can have a negative impact on a wound's healing rate. One of the most important steps in the healing process is keeping damaged tissue free of microbial infection [2].

Continued use of antimicrobial drugs leads to the appearance of antibiotic-resistant bacteria strains and increases the costs of searching for effective antimicrobial agents [3]. Approximately 20,000 resistant genes in bacteria, which have several mechanisms for resistance to standard antimicrobial treatments, have been found [4]. The most common pathogens associated with wound infection in surgeries and burns are Staphylococcus aureus and *Pseudomonas aeruginosa*. Other species, like Enterococci and Enterobacteriaceae, can be found in immunocompromised patients and after abdominal surgery [5].

Plants are considered a source of natural medicinal compounds for humans that play an important role in health care since they are available and less expensive in treatment [6]. Many studies have identified antimicrobial phytochemical compounds such as phenolic acids, flavonoids, tannins, polyketides, terpenoids, glucosides, lignans, saponins, alkaloids, and steroids in plants [7, 8]. The plant seeds have promising antimicrobial activities that could be used as a natural medicinal alternative instead of chemical substances [9]. One of these important medicinal plants is the flax plant (*Linum usitatissimum* L.), cultivated and grown globally; the flax plant has attracted attention to its advantages as a food and medicinal benefit [10]. *Linum usitatissimum* is one of the 180 Linum genus species in the Lamiaceae family [11]. plant's height is (1.2m), and leaves are 20-40mm long and 8mm wide. Have bright blue flowers with (15-22mm) diameter, and the fruit's diameter is (5-9mm) containing glossy brown seeds of (4-7mm) long [12]. Flaxseed is cultivated widely in the Australian, Asian, and Chinese regions [13]. Flaxseed is consumed as a food characterized by its high fiber content, omega-3 fatty acids, phenolic compounds, lignans, and flavonoids [14]. These seeds consist of a large amount of oil, about 35-45%, and 20-25% proteins, in addition to a small quantity of cyanogenic glycosides [15]. The major bioactive constituents in these seeds include triglycerides of α-linolenic acids (52%), oleic acids (20%), and linoleic acids (17%), in addition to the presence of many minerals such as calcium, phosphorus, and magnesium [16].

Many studies revealed important pharmacological properties of flaxseed against various diseases that have antioxidant activity [17], anticancer activity [18], anti-inflammatory [19], antibacterial [20], and antifungal activities [21]. It was revealed that flaxseed plays a role in tumor growth and decreases breast and colon cancer incidence [22]. It also treats respiratory tract infections such as cough, bronchitis, and gastrointestinal infections [12]. Flaxseed oil is an astringent in fungicidal and insecticide lotions [23]. Flaxseed oil has antimicrobial, antioxidant, and wound-healing properties. As well as Omega-3 fatty acids characterized by their anti-inflammatory benefits [24]. Certain studies suggested that the antibacterial activity that the flaxseed possesses is due to its contents of lignans and phenolic acids [25]. The lignan has antioxidant properties and efficacy for treating breast cancer [26]. The ethanol extract of flaxseed has activity against some ovary and endometrial malignant cells due to the content of phenolic compounds [27]. Also, it was confirmed that flaxseed oil has antibacterial activity due to its content of lignans, flavonoids, and phenolic acids [28]. This study aimed to determine the antibacterial activity of flaxseed aqueous, ethanol extracts, and flaxseed oil against different species of bacteria isolated from human wound infections. Furthermore, to investigate all available information about the chemical constituents of flaxseed extracts and assess the various phytochemical compositions.

Materials and Methods

Preparation of flaxseed extracts

The flaxseed and its oil were purchased from a local herbal shop in Basrah Governorate, Iraq. Seeds were cleaned and washed with distilled water and completely shade-dried for one week. Then, these seeds were ground into fine powder using a laboratory grinder. The powder was weighed and stored in a small bottle in a dry place. To make flaxseed aqueous and ethanolic extracts, 50g of seed powder was steeped separately in 500ml distilled water and ethanol. These two extracts were shaken in a rotary shaker for 24 hours and filtered through filter paper (Whatman No.1). A 0.45m micro filter was then utilized in a rotating evaporator at 50°C for additional filtration. The extracts were then kept at 4°C until they were used. These extracts were further diluted with Dimethyl Sulfoxide (DMSO) to achieve varied concentrations (200, 100, 50, and 25g/ml) [29].

Phytochemical screening

Phytochemical screening was carried out to detect the presence of active phytocomponents in flaxseed aqueous and ethanolic extracts such as alkaloids, terpenoids, tannins, flavonoids, glycosides, saponins, steroids, phenolic compounds, proteins, and carbohydrates according to different chemical tests. Flavonoids were detected using the Thamilmaraiselvi *et al.* method, where the appearance of a yellow tint is shown. Steroids were also tested, indicated by a color shift from violet to blue or green [30]. Mayer's reagent was used to detect

alkaloids that appear as cream-colored precipitate [31]. The saponins, whose appearance was creamy and missing small bubbles, were detected according to the Edeoga *et al*. method. Terpenoids were detected using the Salkowski test, which is detected by a reddish-brown interface [32]. According to the method Hanaa *et al.* used to detect tannins and phenolic compounds [33]. As well as the method of Sood *et al.* was also used to detect glycosides [34]. Biuret test was used for the detection of proteins whose presence was confirmed by the formation of violet or pink color. At the same time, carbohydrates were tested using Benedict's test, which formed a reddish-brown precipitate [35].

Bacterial cultures

Flaxseed's antibacterial efficacy was tested against many species of bacteria isolated from wound infections of patients admitted to Al Sadder Teaching Hospital. Wound infections include from the head, trunk, upper and lower extremities (abdomen, leg and foot, hand, and head). Samples were collected from patients with wound infections using a sterile cotton swab and then transported using the transport medium Brain Heart Infusion (BHI); samples were then inoculated into blood agar and McConkey agar using a sterile loop (streaking method) then incubated at 37°C for 24 hours. Double wound swabs were collected from each site simultaneously to decrease the possibility of contamination. Bacterial identification was based on standard microbiological techniques such as Gram stain, colony morphology, and biochemical assays.

Antibacterial activity of flaxseed extracts

The agar well diffusion method tested the antibacterial activity of aqueous and ethanolic extracts and flaxseed oil on bacteria from wound infections. The bacterial species tested included *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella sp.* and *Proteus mirabilis*. A few colonies from these bacterial species were suspended in sterile saline until the turbidity matched McFarland tube number 0.5 (1.5 108CFU/ml). Then, the bacterial inoculum was cultured on Mueller Hinton agar using the streaking method, and each strain of bacteria was inoculated on duplicate plates on agar. Then, a sterile corn borer with a diameter of 6mm was used to punch wells in the agar, where five wells were made in each plate. 50µl of each extract, including aqueous, ethyl, and oil solutions, was added to each well at different concentrations (200, 100, 50, and 25mg/ml), and the same volume of extraction solvent for control was filled in the wells. The antibacterial activities were compared with DMSO as the negative control. Then, all these plates were incubated at 37°C for 24 hours. inhibitory effect was evaluated by measuring the diameter of the bacterial growth inhibitory zone that was produced around the well, and the inhibition zone was measured in millimeters [36].

Findings

Phytochemical screening was carried out on the crude aqueous and ethanolic flaxseed extracts, revealing the presence of many active compounds. These were glycosides, flavonoids, saponins, terpenoids, alkaloids, phenols, tannins, proteins, and amino acids (Table 1). Many of these compounds were present in both extracts, except glycoside and terpenoids, which were not found in the aqueous extract, and saponins, which were not found in the ethyl extract.

Table 1. Phytochemical screening of active compounds for aqueous and ethanolic extracts of *Linum usitatissimum* seeds.

Active compound	Aqueous extract	Ethanolic extract
Tannins	+	+
Phenols	+	+
Alkaloids	+	+
Saponins	+	-
Flavonoids	+	+
Glycosides	-	+
Steroids	+	+
Terpenoids	-	+
Proteins	+	+
Carbohydrates	+	+

Varying degrees of inhibition zones for flaxseed aqueous and ethanolic crude extracts were compared with control solution DMSO, which didn't show an inhibitory effect on all tested bacteria. Results showed that crude ethanolic extract and oil had an inhibitory effect against the growth of all gram-positive and gram-negative bacteria species. It was also revealed that aqueous extract has an inhibitory effect against some gram-positive bacteria, including Staphylococcus aureus and Streptococcus faecalis only. However, no inhibitory effect against gram-negative bacteria was shown (Table 2).

Table 2. The antibacterial activity of crude flaxseed aqueous and ethanolic extracts as well as flaxseed oil against bacteria of wound infection

Microorganisms	Aqueous extract	Ethanolic extract	Flaxseed Oil	DMSO
Gram-positive				
Staphylococcus aureus	Sensitive	Sensitive	Sensitive	Resistant
Streptococcus faecalis	Sensitive	Sensitive	Sensitive	Resistant
Bacillus subtilis	Resistant	Sensitive	Sensitive	Resistant
Gram-negative				
Escherichia coli	Resistant	Sensitive	Sensitive	Resistant
Pseudomonas aeruginosa	Resistant	Sensitive	Sensitive	Resistant
Klebsiella sp.	Resistant	Sensitive	Sensitive	Resistant
Proteus mirabilis	Resistant	Sensitive	Sensitive	Resistant

Aqueous extract showed inhibition zones only for *Staphylococcus aureus* and *Streptococcus faecalis* at 50, 100, and 200mg/ml. All the concentrations of the ethanolic extract showed inhibitory effects against all tested bacteria. The maximum antibacterial effect of ethanol extract on aqueous extract was 200mg/ml. Also, 200mg/ml of flaxseed oil had the most powerful inhibitory effects on all tested bacteria (Table 3).

Table 3. The inhibition zone sizes (millimeters) of different concentrations of flaxseed aqueous extract, ethanolic extract, and oil on isolated bacteria from wound infections

Microorganisms	Aqueous extract				Ethanolic extract				Flaxseed oil			
	200	100	50	25	200	100	50	25	200	100	50	25
Gram-positive												
Staphylococcus aureus	20	14	10	0	26	21	18	12	22	19	10	8
Streptococcus faecalis	17	11	8	0	24	17	12	10	19	16	9	7
Bacillus subtilis	0	0	0	0	20	16	11	9	18	14	10	7
Gram-negative												
Escherichia coli	0	0	0	0	23	16	11	8	14	10	8	0
Pseudomonas aeruginosa	0	0	0	0	21	14	10	9	10	8	0	0
Klebsiella sp.	0	0	0	0	16	11	9	7	12	8	0	0
Proteus mirabilis	0	0	0	0	18	13	7	7	15	9	7	0

Discussion

This study aimed to determine the antibacterial activity of flaxseed aqueous, ethanol extracts, and flaxseed oil against different species of bacteria isolated from human wound infections. Wound infections are still an important problem in human life. The continuing use of antibiotics resulted in the evolution of antibiotic-resistant strains and raised the problem of the side effects of using drugs for a prolonged time. The studies focused on the antibacterial effect of flaxseed against many bacterial species that cause problems in human life. In the current study, we determine the antibacterial activity of the flaxseeds by measuring the inhibitory effect of flaxseeds aqueous, ethanol extracts, and flaxseed oil against the different species of bacteria isolated from wound infections. Also investigated and assessed the various phytochemical compositions of flaxseeds, and the phytochemical analysis of crude aqueous and ethyl flaxseed extracts showed the presence of different active compounds, including glycosides, flavonoids, saponins, terpenoids, steroids, alkaloids, phenols, tannins, proteins, and carbohydrates with few differences. These findings are similar to other studies, which revealed that ethanolic extract lacks saponins, such as Hanaa [33], Amin & Thakur [37], and Alachaher *et al.* [38]. Similarly, other studies confirm that aqueous extract lacks glycoside and terpenoids [12, 20, 33]. The importance of phytochemical compounds in flaxseed is that they can act through various mechanisms and target areas over standard antibiotics to improve efficacy in decreasing resistance development [39]. Many studies have suggested the importance of these phytochemicals against many microorganisms by inhibiting their growth and activity through the action of phenolic acids, flavonoids, tannins, and fatty acids [40, 41]. The phenolic compounds have a role in bacterial DNA breakdown and preventing gyrase activity [42]. Terpenoids stimulate wound healing due to their astringent properties, while fatty acids improve skin moisture to aid healing [43]. Also, oleic and linoleic acids have been suggested to promote wound healing [44].

In our study, flaxseed extracts were tested on *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella sp.*, and *Proteus mirabilis* that were isolated from wound infections. This study included these bacteria because of their important role in causing wound infections. This agreed with other studies that recorded the most prevalent bacterial isolates in wound infections; *Staphylococcus aureus* and *Escherichia coli*, *Proteus species*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [45, 46]. Furthermore, the most common bacterial species in wound infections were gram-positive than gram-negative bacteria [47]. Our study about the crude flaxseed aqueous, ethyl extracts, and oil revealed different inhibitory effects against tested bacteria. The aqueous extract revealed an inhibitory effect against gram-positive bacteria only. Meanwhile, ethanolic extract and the oil extract showed an inhibitory effect on all tested bacteria, with a higher inhibitory effect on gram-positive bacteria than on gram-negative bacteria. All these extracts showed different antibacterial activities regarding their various concentrations; however, their inhibitory effect was found to increase with increased extract concentration.

Several studies agree with these results as they confirmed that the flaxseed has a bactericidal effect against *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* [23]. Also, ethanolic extract of flaxseeds showed promising inhibitory activity against different gram-positive and gram-negative bacteria [10, 12, 37]. In addition, other studies confirmed that flaxseed oil has antibacterial activity against some bacterial species, such as *Staphylococcus aureus* and *E. coli* [46, 48]. Flaxseed oil is considered a good medication that can be used to treat wound infections, especially those caused by bacteria such as *S. aureus* and *K. pneumoniae* [36]. Another study showed that the methanol extract of flaxseed has a higher antibacterial activity than the aqueous extract. The concentration of the extracts, when increased, causes an increase in the size of the inhibition zone [20]. Several studies suggested that *Linum usitatissimum* L. antibacterial activity is based on the presence of natural phenolic compounds, lignans, and fatty acids [40, 49]. Flaxseed contains a high amount of secoisolariciresinoldiglucoside (SDG), a precursor of lignans that may be responsible for the antibacterial action of several flaxseed extracts. The components of lignans have antibacterial effects against gram-positive bacteria [36]. Furthermore,

flavonoids play a role in inhibiting DNA synthesis in *Proteus sp.* and RNA production in *Staphylococcus sp.* [50, 51].

Conclusion

Flaxseed ethanolic extract and flaxseed oil have an inhibitory effect against different species of both gram-positive and gram-negative bacteria isolated from wound infections.

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