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Isolation of antibacterial compounds from *Linum usitatissimum* and evaluation its antibacterial activity

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Abstract

Plants are the main and important source of medicinal agents where traditional treatment plays a major role in health care, and is also a common practice serving as the first aid. Flaxseed or linseed (*Linum usitatissimum*) is one of the important medicinal plant which is cultivated and growing worldwide that has attracted people's interest for its many benefits it offers in human health. The general objective of this study is to isolate antibacterial compounds from *Linum usitatissimum* (Telba) and evaluate its antibacterial activity. The dried seeds were grinded using a laboratory grinder. Dried and powdered seeds (500 g) were extracted using a Soxhlet extractor. Four different extracts of flaxseed were extracted using the chemicals ethanol, petroleum ether, chloroform, and distilled water; then each extracts were concentrated using DMSO solution. In our study, antibacterial properties of four different extracts from *Linum usitatissimum* seeds were screened against three types of Gram-positive and negative bacteria: *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using agar-well diffusion method and comparing their antibacterial activities with the standard antibiotics Ampicillin, Chloramphenicol and Cephalexin. Ethanol extract possessed considerable antibacterial activities compared to the other three extracts, the highest inhibitory effect was observed against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using the extract concentration 100mg/ml. The current result indicates that *Linum usitatissimum* has antibiotic potential for the treatment of pathogenic bacteria. However, our results have not shown positive results for petroleum ether, distilled water, and chloroform solvent extracts of *Linum usitatissimum*.

Keywords: *Linum usitatissimum*, antimicrobial activities, agar-well diffusion, pathogenic bacteria

Introduction

The use of traditional medicine is still widely applicable in Ethiopia, and its acceptability, availability and popularity is no doubt as about most of the populations use it for health care needs. In Africa up to 80% of the population uses traditional medicine to help meet their health care needs (WHO, 2000) [45]. Plants have long been able to provide humans a source of medicinal agents with natural products which can either be primary or secondary metabolites, which in turn could serve as the source of all drugs (Balandrin *et al.*, 1993) [6].

In developing countries, plants are the main and important source of medicinal agents where traditional treatment plays a major role in health care, and is also a common practice serving as the first aid (Farnsworth, 1994; Rabe and van Staden, 1997). The rural population of a country does not use modernized drugs, which makes them more disposed to traditional ways of treatment because it is easily available and cheaper in cost (Brantner and Grein, 1994) [10]. Herbal therapy being still an unwritten field of science, is well established and practiced in some cultures and traditions, by becoming a way of life in almost 80% of the people in rural areas, especially those in Asia, Latin America and Africa (Jäger *et al.*, 1996).

The traditional use of medicinal plants for the manufacture of commercial drugs and its substitutes has been predominant from ancient times. These drugs help in the treatment of different form of ailments caused by bacterial and fungal pathogens and in other important health-related activities (Cowan, 1999) [13].

Flaxseed or linseed (*Linum usitatissimum*) is one of important medicinal plant which is cultivated and growing worldwide has attracted people's interest for its many benefits it offers in human health (Rubilar *et al.*, 2010) [27, 41]. Lipid, fiber and protein are ingredients of the seed contents of Flaxseed (Anjum *et al.*, 2003) [4]. Mucilage is another important component of flaxseed, in which its composition presents a heterogeneous mixture of polysaccharides.

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The major product gained from flaxseed is oil which is rich in omega 3 fatty acids, similar to fish oil, and helpful in preventing cardiovascular diseases and cancer (Gutiérrez *et al.*, 2010) [27, 41].

The flaxseed (*Linum usitatissimum*) is the seed from the flax plant, an annual herb growing to a height of 0.3-1m, which belongs to the *Linaceae* family containing more than 200 species. The plant is native to India and the eastern Mediterranean but distributed throughout the world including Canada, China, United States (Montana, Dakota, Minnesota, etc.), Ethiopia and all over Europe (Worku *et al.*, 2015) [46]. It is an erect, herbaceous annual whose branches are found above the main stem. The word Flaxseed comes from Latin meaning "very useful", and it has brown and golden varieties. The shape of flaxseed is flat or oval up to 4–6 mm size with a pointed tip. Two types of *L. usitatissimum* are cultivated: firstly the linseed type, grown for oil extraction from the seed, is a relatively short plant which produces many secondary branches compared to the flax type, secondly, grown for the fiber extraction from the stem, which is taller and is less branched (Oomah *et al.*, 2007).

It is also cultivated for its flexible fibers. The plant has well known medicinal properties. Historically, linseed oil, derived from flaxseed, has been used as a topical demulcent and emollient, as a laxative, and as a treatment for coughs, colds, and urinary tract infections. This flaxseed plant is the rich source of the bioactive components phenylpropanoids- the secondary metabolites exhibiting the strong antioxidant properties and thus possessing the inhibitory effect on bacteria, viruses and fungi (Czemplik *et al.*, 2011) [16]. Seeds of the plant contain 35-45% oil comprising mainly linoleic and linolenic acids and 20-25% protein. The seed also contains cyanogenic glycosides (prussic acid) in small quantities, which stimulate respiration and improve digestion (Coşkuner and Karababa, 2007) [12].

Flax (*Linum usitatissimum*) is cultivated for the production of textile fiber, seed and flaxseed (linseed) oil. Until a few years ago, flax was cultivated in Los Lagos Region, Southern Chile, mainly as a raw material for textile industry. Nowadays, flax is cultivated in Araucanía Region for oil extraction. Studies have shown that crop yield in this region is higher because of its suitable soil and climate characteristics. Flax is best suited for fertile, fine textured and loamy soils. An important factor is the amount of rainfall during the growing period. Adequate moisture and relatively cool temperatures seem to favor both oil content and oil quality, to a great extent during the period from flowering to maturity. The seed is located in the extremities of the branches in round capsules, each of which contains from one to ten seeds. It is well known that flax seeds are a source of high quality proteins, soluble fiber and a high content of polyunsaturated fatty acids (Pradhan *et al.*, 2010) [40].

Material and methods

Extraction procedure

Flax seeds were obtained from the local market. The seeds were washed, cleaned of extraneous matter and shade dried completely. The seeds were dried at room temperature under shade for a period of one week. The dried seeds were grinded using a laboratory grinder. Dried and powdered seeds (500 g) were extracted using a Soxhlet extractor with solvents of increasing polarity beginning with petroleum ether followed by ethanol then distilled water and finally chloroform, each extraction was put on the shaker for 48 hours (Ashnagar *et al.*, 2005) [5]. By using Watman filter paper, the solvents were filtered to get the purified one. The solvents were removed using a rotary vacuum evaporator at 60°C for petroleum ether,

71°C for ethanol, 100°C for water and 45°C for chloroform to give concentrated extracts which were frozen and freeze-dried using lyophilizer until use.

Preparation of extract concentrations

DMSO (Dimethyl sulfoxide) solution was prepared by mixing 5 ml of DMSO in 100 ml of distilled water. Two doses were prepared using 10mg of each extract (petroleum ether, ethanol and chloroform) with 10ml DMSO solution and 5mg of each extract with 10ml of DMSO solution to give an extract concentration which was used as a standard concentration (Erturk *et al.*, 2006) [20].

Preparation of media

The required quantities of Muller Hinton agar (38g 1000ml-1) is prepared by dissolving it in distilled water in conical flasks and heated on a hot plate. Media was sterilized in an autoclave at 15 psi pressure and 121°C for 15 min. After sterilization, Muller Hinton agar media was poured aseptically into sterilized Petri dishes in a laminar flow hood. The media was allowed to be solidified in the Petri dishes for about 20 min and then allowed for inoculation of bacteria. All the steps were performed in sterile environment in order to prevent contamination.

Microbial cultures

Bacterial strains tested included the gram positive strain: *Staphylococcus aureus* and the gram negative strains: *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* which all had been kindly provided from Gondar University Hospital microbial laboratory. These bacteria would further be identified by using characteristic features including effectiveness of the antibiotics and clear zone of the cultural tests by comparing with the standard antibiotic effects on the bacterial cultures.

Preparation of inoculums

All bacterial strains were cultured overnight in Nutrient broth, incubated at 37°C and used as inoculums. The turbidity of the suspensions was adjusted to (1.5–3×10⁸ cells/ml) in comparison with McFarland turbidity standard. Serial dilutions were prepared from 10⁻¹–10⁻⁷ using normal saline solution in order to decrease the number of bacteria growing on the media.

Antibacterial activity

The antibacterial tests were performed using agar-well diffusion method described by (Bauer *et al.*, 1966) [8]. The bacterial inoculums (1ml) were evenly spread onto the surface of the agar plates using sterile swab sticks. Wells (6 mm diameter) were punched in the plates using a sterile stainless steel borer. One ml of each extract concentrations with two doses (5gm/10ml and 10gm/10ml) were added to the first two wells. Commercial antibiotics Ampicillin 250mg, Cephalexin 250mg and Chloramphenicol 250mg were purchased from a local pharmacy, each antibiotic capsule (250mg) was dissolved in 10ml of ethanol (70% or absolute depending on antibiotic) to produce antibiotic solutions with a concentration 25mg/cm³ as described by (Adomi, 2006) [1]. One ml of each standard antibiotic solutions were filled in a third well and used as a positive control, and 1ml of DMSO was used as negative control in a fourth well. Diffusion of the extracts and antibiotics was allowed at room temperature for 30 minutes. The agar plates were then incubated at 37°C for 24 h. The plates were observed for the presence of a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity expressed in terms of the average

diameter of the zone inhibition in millimeters. The absence of a zone inhibition was interpreted as the absence of activity.

Result and discussion

Extract results

Flaxseed’s powder soaked with the four chemical solvents upon filtration with the Watman filter paper resulted in different states. The extracts of petroleum ether and ethanol were easily filtered through the filter paper, while the other two extracts, distilled water and chloroform, and were unable to pass through the filter paper easily. To overcome this problem, an alternative gauze was used. Evaporation of each solvent with rotary evaporator came up with the required pure extract. This pure extract resulted in a gel form, except for the distilled water extract which was found to be in a powder form.

Antibacterial evaluation of *Linum usitatissimum* extracts

The antibacterial activity of each extract with respect to the selected pathogenic bacteria was evaluated by observing the presence of a clear zone. Among the four extracts, ethanol extract happened to show good antibacterial activity. This antibacterial activity was especially seen against *Ps. aeruginosa* and *K. pneumonia* compared with the commercial antibiotics ampicillin, cephalixin and chloramphenicol. The calculated inhibition zone reached 10mm and 9mm for *Ps. aeruginosa* and 12mm and 11.5mm for *K. pneumonia* in diameter using the ethanol extract concentration of 100mg/ml. However, the rest of the three extracts, did not show any antibacterial activity against all tested bacteria, as they did not show any inhibition zone (Table1).

Table 1: Antibacterial activity of different extracts from *Linum usitatissimum* L. seeds.

Microorganisms	Extracts	Zone of inhibition (mm)				
		Extract concentration		Positive control		
		10gm	5gm	Cephalexin	Ampicillin	Chloramphenicol
S. aureus	P. ether	-ve	-ve		18mm	
	D. water	-ve	-ve			
	Ethanol	-ve	-ve			
	Chloroform	-ve	-ve			
K. pneumonia	P. ether	-ve	-ve	19.2mm		
	D. water	-ve	-ve			
	Ethanol	12mm	11.5mm			
	Chloroform	-ve	-ve			
Ps. Aeruginosa	P. ether	-ve	-ve			17.5mm
	D. water	-ve	-ve			
	Ethanol	10mm	9mm			
	Chloroform	-ve	-ve			

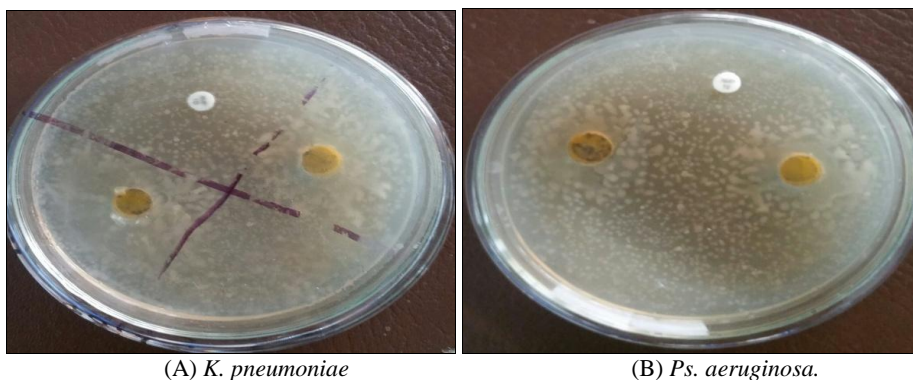


Fig 1: Image of antibacterial activity of the ethanol extracted samples of *L. usitatissimum* against

Standard antibiotics cephalixin, chloramphenicol and ampicillin were effective against all the selected bacteria and showed a zone of inhibition of 19-25mm. The result of the

investigation showed that the seed extract have good antibacterial activity against, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

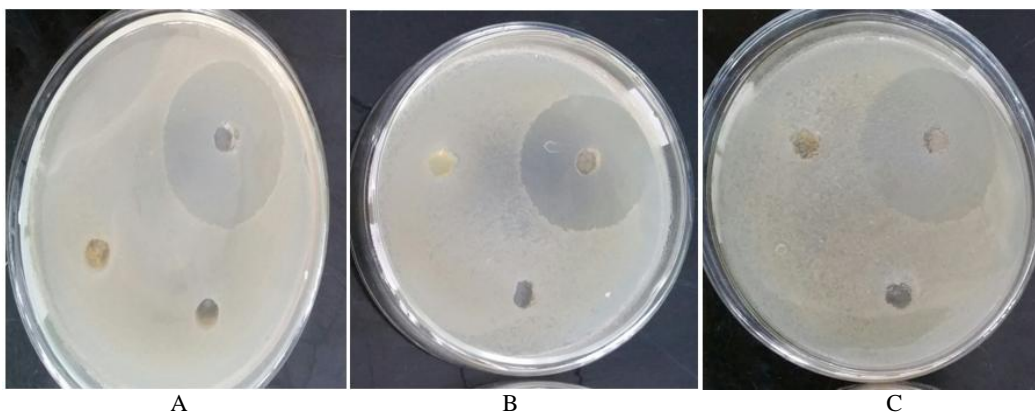


Fig 2: Antibacterial activity of (A) chloroform, (B) petroleum ether and (C) distilled wate against *Ps. aeruginosa*.

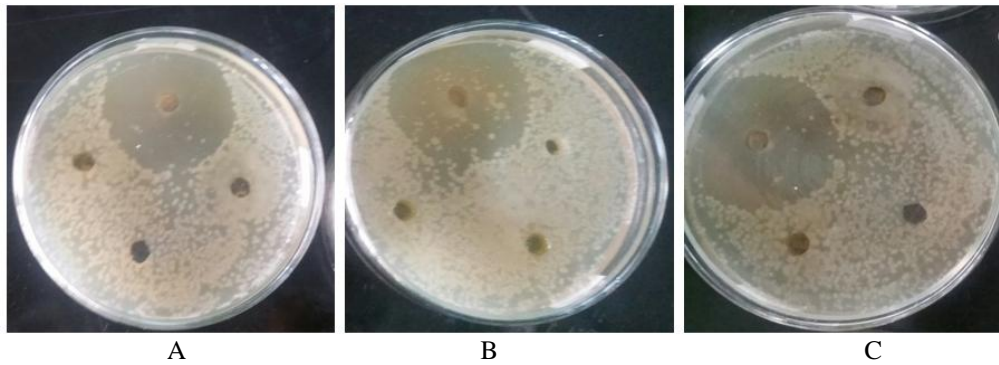


Fig 3: Antibiotic activity of petroleum ether, distilled water and chloroform extracts against *S. aureus*.

Discussion

The present study was conducted to evaluate antibacterial effects of *Linum usitatissimum* extracts to both gram positive and gram negative bacteria. The study results indicated that ethanol extracts of *Linum usitatissimum* shows antibacterial activity to gram negative bacteria of both *Klebsila pneumonia* and *Pseudomonas aeruginosa*. The effect of antibacterial activity was detected by the formation of clear zone of inhibition of 10.2-20.5mm (Kreander *et al.*, 2006). The observed antibacterial activity of extract is due to Lignan and alpha linoleic acid compounds.

The study results revealed that ethanol extract possessed good inhibitory activity against *B. cereus* compared with the antibiotics ampicillin, cephalixin and tetracycline, the calculated inhibition zone reached 22.6mm in diameter using the extract concentration 200mg/cm³, *S. aureus* showed moderate sensitivity towards the ethanol extract using the highest extract concentration compared with ampicillin and tetracycline, *Ps. aeruginosa* and *K. pneumoniae* revealed weak sensitivity towards the extract compared with the tested antibiotics. The study results proved the suitability of ethanol in dissolving active components from plants in addition to natural products (Herrero *et al.*, 2006). Ethanol extract activity against tested bacteria can be attributed to the linseed content of mucilage, gum, wax, cyanogenic and glycosides. Wax and cyanogenic glycosides have been reported to contain compounds that have antifungal and antibacterial effect (Aboaba *et al.*, 2006; Reid *et al.*, 2005),

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