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Physicochemical characteristics and antimicrobial of composition from *linum usitatissimum* L seeds oil

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Abstract
Linum usitatissimum L a member of *Linaceae* family, which is used for various medical purposes. The aims of this study is the isolation of oil, testing with some microbes and measure some physicochemical properties. The seeds of *Linum usitatissimum* L was collected from the Omdurman and air-dried at room temperature, the oil extract of seed was prepared using soxhlet extraction with n-hexane, and analyzed using GC-MS Spectroscopy. The oil extract was tested for antimicrobial activity against 6 stander human pathogens *Pseudomonas aeruginosa* (P.s), *Bacillus subtilis* (B.s), *Staphylococcus aureus* (S.a), *Escherichia coli* (E.C), *Condidaalbacans* (Ca), and *Aspergillusniger* (A.s), and measured some physicochemical properties such as acid value, saponification value, and peroxide value. GC-MS characterization of fatty acid profile revealed the dominant fatty acid in 9,12,15-Octadectrienoic acid (51.46%), followed by oleic acid 9,12-Octadecdienoic acid (21.40%), Hexadecenoic acid (12.61%) and Methyl stearate (10.14%). Approximate of physiochemical properties of extracted oil revealed acid value (0,810 mg KOH/g of oil), saponification value (188.7), and peroxide value (0.99 meqO_2/kg). The oil showed in active on all the test organisms except *Candida albicans.*

Keywords: *Linum usitatissimum* L, linseed, *Linaceae*, GC-MS, physicochemical

Introduction
Linum usitatissimum L. It is known as the common name Flax, and "Kettan" in some Arab countries, it is an annual plant belonging to the genus Linum and the family Linaceae [1]. Linseed oil is derived from the seeds of *Linum usitatissimum* L., a plant widely cultivated in Europe for fiber or oil for industrial use [2]. The most important linseed producing countries are Canada, Argentina, USA, China, India and Europe [3]. The height of plant varies from 30 to 36 inches and has small, narrow alternate, lance leaves that are less than an inch long. Stems is slender and very fibrous stems are branched near the base of plant, the leaves having three veins, up to 4 cm long and 4 mm wide, and its bright blue flowers are up to 3 cm in diameter [4, 5]. The flowers of most cultivated varieties range in color from deep to pale shades of blue. Some garden varieties have white, violet, pink, or red blossoms. Flax seeds are flat and oval with pointed tip and their color varies from dark brown to yellow [6]. The life cycle of the flax plant consists of a 60-to-80-day vegetative period, 25-to-40-day flowering period and a maturation period of 40 to 60 days [7]. Humans have consumed flaxseed since the beginnings of the earliest civilizations. It was used for medical purposes in ancient Egypt and Greece, mainly to relieve abdominal pains and also as energy source [9]. In addition to edible uses of this oil, it is known as an anti-inflammatory [8,9] antioxidant [10] and analgesic [9], anti-fibrosis drug [11], anti-diabetic stabilizing blood-sugar levels, antiviral, bactericidal, and ant atherosclerotic agent is known [9]. Oil Therefore, due to the mentioned beneficial properties, it is used in several studies on a variety of subjects such as arthritis [10], dermatologic complaints [12] breast cancer [13] and even keratoconjunctivitis [14],The topical use of linseed oil has been approved for a variety of skin disorders [15]. For instance, the Brazilian national pharmacopoeia has approved its topical administration in cases with pruritus, and in patients of burn [10]. Compared to a control group of postmenopausal women with newly diagnosed breast cancer, those who consumed 25 g ground flaxseed per day for approximately 32 days showed decreased tumor cell proliferation, and increased apoptosis

at the time of surgery [16, 17].

2. Material and methods

2.1. Sample collection and preparation

The Seed of *Linum usitatissimum* L. were purchased from the Omdurman market. The plant was kindly authenticated by Institute of Aromatic and Medicinal Plants- Khartoum, Sudan. The plant seeds were dried in the shade. After drying, the seeds were ground well into fine powder using mechanical blender, and the powder was transferred into airtight containers with proper labeling for future use. Extraction of oil Powdered seeds of *Linum usitatissimum* L. (200 g) were exhaustively extracted with 500 ml of n-hexane (soxhlet) at 60 °C for 6 hours. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4 °C for further manipulation.

2.2. Test organisms

Linum usitatissimum L. oil was tested for antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method with two Gram-positive bacteria *Staphylococcus aureus*, *Bacillus Cereus*, and two Gram - negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and two fungal strains *Candida albicans*, *Aspergillus* Niger taking ciprofloxacin, norfloxacin, nystatin as standard drugs.

2.3. Esterification of oil

Methanolic solution of sodium hydroxide was prepared by dissolving (2 g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic sulphuric acid was prepared by mixing (1 ml) of concentrated sulphuric acid with (99 ml) methanol.

The oil (2 ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid. The tube was stoppered and shaked vigorously for five minutes and then left overnight. (2 ml) of supersaturated sodium chloride were added, then (2 ml) of normal hexane were added and the tube was vigorously shaked for five minutes. The hexane layer was then separated. (5µl) of the hexane extract were mixed with 5ml diethyl ether. The solution was filtered and the filtrate (1µl) was injected in the GC-MS vial.

2.4. GC-MS analysis

Linum usitatissimum L. fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used. Helium (purity; 99.99%) was used as carrier gas. Oven temperature program is given in Table 1, while other chromatographic conditions are depicted in Table 2.

Table 1: Oven temperature program

Rate	Temperature(C)	Hold time (mim.$^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 2: Chromatographic conditions

Column oven temperature	1300.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

2.6. Physicochemical properties

Some of physicochemical properties of *Linum usitatissimum* L fixed oil such as acid value, saponification and peroxide values, were estimated according to the AOCS method [18].

2.7. Antimicrobial assay

2.7.1. Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37 °C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 108-109 colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37 °C for 24 hours.

2.7.2. Preparation of fungal suspensions

Fungal cultures were maintained on dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

2.7.3. Testing for antibacterial activity

The cup-plate agar diffusion method was adopted, with some minor modifications, to assess the antibacterial activity. (2 ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45 °C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were

cut using sterile cork borer (No 4), each one of the halves was designed for one of the test solutions. Separate Petri dishes were designed for standard antibacterial chemotherapeutics (Ciprofloxacin, Norfloxacin, and Nystatin).

The agar discs were removed, alternate cups were filled with 0.1 ml samples of each test solution using adjustable volume micrometer pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 °C for 24 hours.

The above procedure was repeated for different concentrations of the test solutions and the standard chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

3. Results and Discussion

3.1. Physicochemical properties

The *Linum usitatissimum* L. Seeds oil had yellow colour and a very characteristic flavor. The oil was also stable at the room temperature. The physical and chemical characteristic of the seed oil are summarized in Table 3. The physical results from the oil indicated that these properties of the seed oil are similar to oils rich in linoleic acid.

Table 3: Physical and Chemical Characteristics of *Linum usitatissimum* L. Seeds Oil

Properties	Estimated value
Acid value (mgKOH/g)	0,810
Saponification value (mgKOH/g)	188.7
Peroxide value (mmolO_2/kg)	0.99

The GC-MS analysis of *Linum usitatissimum* L. fixed oil:

GC-MS analysis of *Linum usitatissimum* L. oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

3.2. Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 26 components (Table 4).The typical total ion chromatograms (TIC) is depicted in Fig.1. Compound 9, 12, 15-Octadectrienoic acid, methyl ester was found to be in the highest concentration (51.46%) followed by 9, 12-Octadecdienoic acid (Z, Z), methyl ester (21.40%), Hexadecenoic acid, methyl ester (12.61%) and Methyl stearate (10.14%), other compounds were found in trace amount shown in Table (3). Either one or all the identified compounds may be responsible for the antimicrobial activity of the oil.

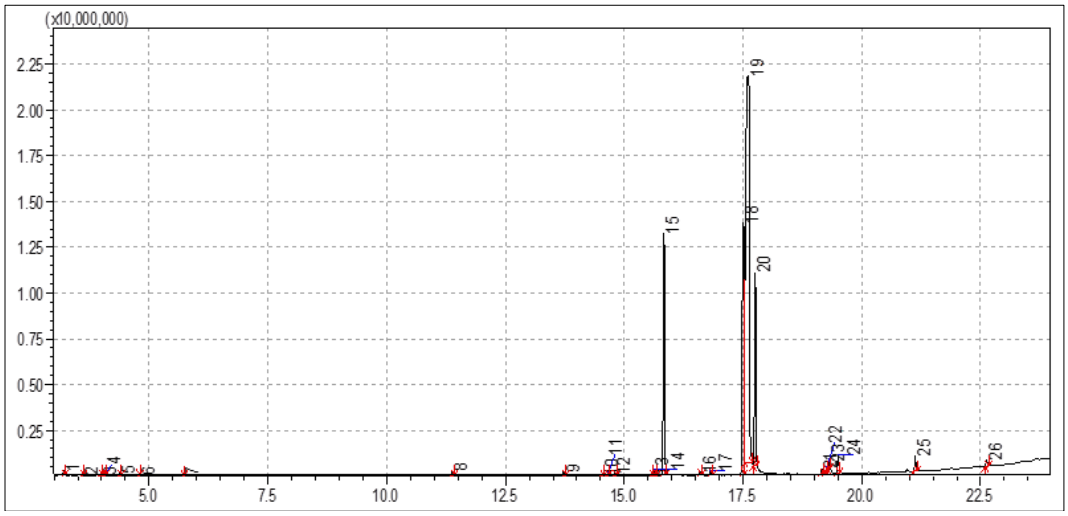

Fig 1: GC-MS Spectrum of *Linum usitatissimum* L. seeds oil.

Table 4: Constituents of *Linum usitatissimum* L. oil

Peak No.	Name	R. Time	Area	Area %
1	Nonane	3.198	464015	0.22
2	Cyclohexane, proyl ester	3.601	33867	0.02
3	Benzene, 1-ethyl-3-methyl -	3.977	111694	0.05
4	Benzene, 1,2,3-trimethyl-	4.060	58520	0.03
5	Benzene, 1,2,4-trimethyl-	4.373	206817	0.10
6	Mesitylene	4.783	38090	0.02
7	Undecane	5.717	25508	0.01
8	Butylated hydroxytolune	11.371	458925	0.22
9	Methyl tetradecanoate	13.725	326664	0.15
10	5-Octadecenoic acid, methyl ester	14.538	37801	0.02
11	6-Octadecenoic acid, methyl ester,(Z)-	14.640	31074	0.01
12	Pentadecenoic acid, methyl ester	14.801	166231	0.08
13	9-Hexadecenoic acid, methyl ester, (Z)-	15.596	144368	0.07
14	7-Hexadecenoic acid, methyl ester, (Z)-	15.636	600841	0.28
15	Hexadecenoic acid, methyl ester,	15.838	26719097	12.61
16	Cis-10-Heptadecanoic acid, methyl ester	16.601	251185	0.12

17	Heptadecanoic acid, methyl ester	16.803	471636	0.22
18	9,12-Octadecdienoic acid (Z, Z), methyl ester	17.508	45353650	21.40
19	9,12,15-Octadectrienoic acid, methyl ester	17.599	109051586	51.46
20	Methyl stearate	17.758	21491529	10.14
21	Methyl 8,11,14-heptadecatrienoate	19.155	668405	0.32
22	2(1H)-Naphthalenone, octahydro-3-methylester	19.263	571823	0.27
23	11-Eicosenoic acid, methyl ester	19.304	558509	0.26
24	Methyl 18-methylnonadecanoate	19.501	1643327	0.78
25	Methyl 20-methyl-heneicosanoate	21.122	1629248	0.77
26	Tetracosanoic acid, methyl ester	22.625	818160	0.39
Total			211932552	100%

3.3. Antibacterial activity

The oil was screened for antimicrobial activity against six standard organisms. The average of the diameters of the growth of inhibition zones are depicted in Table (5). As shown in Table (5), the *Linum usitatissimum* L. seeds oil were not effective against most of the microorganism except *Candida albicans* is partially effective.

Table 5: Antibacterial and antifungal activity of *Linum usitatissimum* L. oil standard drugs:

Name of compounds	Minimal inhibition concentration (µg mL-1					
	Gram-positive		Gram-negative		Fungal species	
	Sa	Bs	Ec	Ps	Ca	An
Oil	-	-	-	-	12	-
Ciprofloxacin	50	50	25	25	-	-
Norfloxacin	10	10	10	10	-	-
Nystatin	-	-	-	-	100	100

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

4. Conclusion

Present study of the *Linum usitatissimum* L. seeds oil indicated that it contains biologically active compounds. The properties of these compounds probably contribute, at least to some extent, to the pharmacological and traditional medicinal uses of the *Linum usitatissimum* L. seeds oil. Further separation and identification of compound present in it may give new biologically active compounds, which can be used as lead compounds in future.

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