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Synthesis, antibacterial activity of new fatty acid thiosemicarbazide from *linum usitatissimum* (linseed) seed oil and it's characterization by X-RD

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ABSTRACT

Linum usitatissimum (Linseed) seed oil is yellow coloured viscous liquid having high value of iodine value indicate higher content of unsaturated fatty acids. A synthesis of new fatty acid thiosemicarbazide derivative from Linum usitatissimum (Linseed) seed oil .The purpose of this study was investigating experimental the possible extraction of Linum usitatissimum (Linseed) seed oil by using n-hexane . Preparation of mixed fatty acids from extracted oil and then synthesis of fatty acid thiosemicarbazide derivative . The infrared spectra and X-ray diffraction of fatty acid thiosemicarbazide was studied . The antibacterial activity of fatty acid thiosemicarbazide was evaluated . The bacteria B. cerus and E. coli was found to be more active and S.aureus was found to be less active.

Key words: *Linum usitatissimum*, Thiosemicarbazide, Antimicrobial sample, X-RD.

INTRODUCTION

Thiosemicarbazone derivative are of special importance because of their versatile biological and pharmacological activities. It have found application in drug development for the treatment of central nervous system disorders of bacterial infection as well as analgesic and antiallergenic agent [1]. Thiosemicarbazones are potent intermediate for the synthesis of pharmaceutical and bioactive materials and thus they are used extensively in the field of medicinal chemistry. Moreever thiosemicarbazones have found their way into almost every branch of chemistry, commercially they are used as dyes, phoyographic films, plastic and in textile industry [2] Traces of interest date back to the beginning of the 20th century but the first reports on their medical applications began to appear in the Fifties as drugs against tuberculosis and leprosy [3,4]. In the Sixties their antiviral properties were discovered and a huge amount of research was carried out that eventually led to the commercialization of methisazone, to treat smallpox [5]. In this period one of the first antitumor activity results was published [6].Recently Triapine®(3-aminopyridine-2-carboxaldehyde thiosemicarbazone) has been developed as an anticancer drug and has reached clinical phase II on several cancer types ,[7,8]. Thiosemicarbazone derivatives are of special importance because of their versatile biological and pharmacological activities. Thiosemicarbazone derivatives have found application in drug development for the treatment of central nervous system disorders, of bacterial infection, as well as analgesic and antiallergic agent. Thiosemicarbazones are potent intermediates for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in the field of medicinal chemistry. Moreover, thiosemicarbazones have found their way into almost every branch of chemistry; commercially they are used as dyes, photographic films, plastic and in textile industry.

Over the years, thiosemicarbazone derivatives have demonstrated wide range of biological activity *viz.* antimicrobial [9-14], antitumor[15-16], sodium channel blocker [17], anticancer [18-19], antitubercular[20],antiviral

[21],antibacterial [22], antimalarial [23]. Thiosemicarbazides posses useful pharmacological and corrosion inhibition properties. They have been frequently employed for the quantitative determination of inorganic ions [24-26].

Authentication of vegetable oils is of great importance, especially nowdays due to the expending demand, Determination of adulteration and characterization are based on the analysis of major [27-31], minor [32-33] or both major and minor [34-35] compounds of the oils.

Major compounds are triacylglycerol (TAGs) present as 95-98 % & minor compounds are different varities of compounds present as 5-2 % such as wax, esters, hydrocarbons, phenolic derivatives etc . The fatty acid composition of linseed oil is dominated by C18 fatty acids C18:2 (16 % of oil) C18:3 (50% of oil) . Typical % of fatty acid content of linseed oil is C16:0 6% Palmitic C18:0 2.5% Stearic Acid C20 0.5% Arachidic acid C18:1 19% Oleic acid C18:2 24.1% Linoleic acid C18:3 47.4% Linolenic 0.2% Other . linseed oil consists chiefly of three glycerides, called, respectively, linolein, linolenin, and olein. [33] . The fatty acid composition of linseed oil is dominated by C18 fatty acids C18:2 (16 % of oil) C18:3 (50% of oil) . Typical % of fatty acid content of linseed oil is C16:0 6% Palmitic C18:0 2.5% Stearic Acid C20 0.5% Arachidic acid C18:1 19% Oleic acid C18:2 24.1% Linoleic acid C18:3 47.4% Linolenic 0.2% Other . linseed oil consists chiefly of three glycerides, called, respectively, linolein, linolenin, and olein. A small, but variable, amount of free fatty acids, such as palmitic and arachidic, is also present. Flaxseed oil is the richest sources of Omega 3 essential fatty acids. Omega-3 fatty acids have been shown to reduce inflammation and help prevent certain chronic diseases such as heart disease and arthritis. These essential fatty acids appear to be particularly important for cognitive and behavioral function as well as normal growth and development. Major health claims for flaxseed and flax oil arise from the fact that these products contain high levels of ALA. ALA can be converted by the body into two different long chain omega-3 fatty acids. Flaxseed oil has been proven to lower cholesterol [36] , which decreases the risk of heart disease. Flaxseed oil has superb anti-inflammatory properties. It inhibits inflammatory reactions that cause blood circulation problems and protects against high blood pressure as a result. The oil not only reduces inflammation in the arteries, but also in joints, the digestive system, kidneys and skin. Flax seeds are high in fibre and provide relief for constipation and haemorrhoids. Flaxseed oil may help to prevent swelling and inflammation of the prostate that tends to enlarge with age in males. It is a rich source of lignans, which seem to have an advantageous effect on hormones with its anti-viral, anti-bacterial, anti-fungal and anti-cancer properties. Lignans may protect against breast, prostate, colon and perhaps even skin cancer. The hormone-balancing lignans can help stabilize the female hormones, that can have beneficial effects on the menstrual cycle.

Essential fatty acids Important to every cell in the body for normal growth, especially of the blood vessels and nerves and to keep the skin and other tissues youthful and supple through their lubricating quality. These nutrients are invaluable for the production and movement of energy throughout the body. They regulate the transportation of oxygen and are vital in maintaining the integrity of cell structure. The essential fatty acids are all polyunsaturated and have the unique ability to lower cholesterol levels of the blood.Essential fatty acids are essential for the function and structure of the brain and improve cognition, memory, moods and concentration.. It has excellent skin-healing properties and is often used in the treatment of eczema, psoriasis and even acne. Not only is it good for skin, but it nourishes hair and nails and stimulates the healthy growth . Flaxseed and its derivative flaxseed oil (or linseed oil) are rich sources of the essential fatty acid alpha-linolenic acid (ALA), which is a biologic precursor to omega-3 fatty acids such as eicosapentaenoic acid. Although omega-3 fatty acids have been associated with improved cardiovascular outcomes, evidence from human trials is mixed regarding the efficacy of flaxseed products for coronary artery disease or hyperlipidemia (high lipid levels). Fatty acid derivative anticonvulsants appear to increase the availability of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter. They have several mechanisms of action. They have inhibitory action against GABA transaminase, which breaksdown GABA. This leads to increased concentration of GABA in the synapses.Linseed and its oil have lots of medicinal applications. These medicine are practiced in Rural and Adivasi communities of Maharashtra .There are many Institutions abroad taking keen interest in Herbal Medicines of India origin. In order to get rid of above mentioned problems, the present work is undertaken. Fatty acid thiosemicarbazide synthesized from *Linum usitatissimum* (Linseed 0 seed oil and Characterized by IR and X-RD techniques.

Linum usitatissimum is a small herbaceous annual plant . The Common name : Flax, Family: Linaceae , Genus: Linum , Species: usitatissimum .

MATERIALS AND METHODS

Collection of plant material

The dried *Linum usitatissimum* (Linseed) seeds were obtained from local market in Ahmednagar, Maharashtra,India. They are dried in room,clean and stored in sealed vessel wrapped with a polyethylene bag at 4^0C .

Extraction of oil

After cleaning and removal of the sand and foreign materials, the dried *Linum usitatissimum* (Linseed) seeds were ground to a fine powder using a grinde. The oil was extracted with n-hexane (1:4w/v) by continous extraction in a soxhlet apparatus for 12 hours. The solvent was evaporated at 40 ^{0}C to dryness. The extracted oil was stored in sealed and dark bottles. Their analysis for physic-chemical characteristics by standard BIS methods [37] gave specific gravity at 25^0C 0.8325 ,refractive index at 25 ^{0}C 1.4525 , acid value (mg KOH /g of oil) 1.05, iodine value (g/100g of oil) 163.5 and saponification value (mg KOH / g of oil) 191.2 . All the other chemicals used in the study were of laboratory grade and were used without any modification.

Preparation of mixed fatty acids from oil

Mixed fatty acids from *Linum usitatissimum* (Linseed) seeds oils were obtained by saponification method in which 25 g oil was taken in 250 ml round bottom flask and 30 % alcoholic NaOH was added. The contents were refluxed for 3 hrs on stirring water bath. At the end of the reaction, the excess alcohol was distilled off and soap was dissolved in hot water. Then the fatty acids were liberated by acidifying the soap solution with 1:1 H_2SO_4 (added till development of red colour in methyl red), washed and dried over anhydrous sodium sulphate.

Preparation of fatty acid thiosemicarbazide (FATSC)

Fatty acid (1.0 g) were dissolved in 2 ml of methanol and the solution was boiled gently for 20 min. To a solution of fatty acid 2.0 g thiosemicarbazide in methanol (20 ml) was added with constant stirring . This mixture was refluxed for 3 hrs on stirring water bath and kept it overnight. Crystal was filtered washed and dried. These crystals were recrystallised from alcohol

Infrared spectra of FATSC

The infrared spectra of fatty acid thiosemicarbazide(FATSC) was taken in the range of 4000 cm^{-1} to 750 cm^{-1} on perkin Elmer 221 IR Spectrophotometer using KBr pellet technique. The characteristic bands observed are as in Table 1. Fig 1. Shows IR spectra of fatty acid thiosemicarbazide .

X-RD spectra of FATSC

X-RD spectra of fatty acid thiosemicarbazide was taken on PW 3710 diffractometer using CuK_2 radiation (Y= 1.54060) The X-RD diffraction of fatty acid thiosemicarbazide was recorded at angle 2Ɵ from 18.5230 to 36.6561. The data of X-ray diffraction of fatty acid thiosemicarbazide were presented in Table 2. And X-ray spectrum in fig 2. For the determination of structure Hesse-Lipson procedure is used [38].

Antibacterial Activity of FATSC

Antibacterial activity of fatty acid thiosemicarbazide of *Linum usitatissimum* (Linseed) seeds oil was analyzed. Table 3. Two Gram-positive bacteria, Staphylococcus aureus , Bacillus cereus and one Gram-negative bacteria Escherichia coli were used. Inoculum size was adjusted to 1 to 2 $x17^7$ CFU (Colony Forming Units) / ml by serial dilution with sterilized nutrient broth media . Nutrient agar (PH 7.2) was used for routine susceptibility testing of nonfastidious bacteria . Stock solution of 1000mg / ml was prepared in 20 % v/v water in DMSO . Using the stock solution, 6000 mg/ ml , 4000 mg / ml , 2000 mg / ml and 1500 mg / ml solutions were prepared from which 100ml solution was taken for assay . Ciprofloxacin was used as a standard . 20 % v/v WFI in DMSO was used as a control . Antibacterial assay was carried out by agar Well Diffusion Method . After 16 to 18 hours of incubation , each plate is examined.

RESULTS AND DISCUSSION

Linum usitatissimum (Linseed) seed oil is yellow coloured viscous liquid at room temperature. The seed oil indicate high content of polysaturated fatty acids . The seed oil has Specific gravity 0.8325 and refractive index 1.4525. The oil has acid value 1.05 (mg KOH / g of oil) , This value measures of the amount of free fatty acids present. .The free fatty acid value suggested that *Linum usitatissimum* (Linseed) seed oil is stable . The saponification number is 191.2 (mg KOH / g of oil) & iodine value 163.5 (mg /100 g of oil) indicate a high level of unsaturated fatty acid is an assest in nutritionas high content of saturated fatty acids is implicated in cardiovascular diseases. Experimentally it found that *Linum usitatissimum* (Linseed) seed oil is used a medicinal important . Infrared spectra of fatty acid of thiosemicarbazide of *Linum usitatissimum* (Linseed) seed oil shows that at 750 cm^{-1} strong CH_2 , 850 cm^{-1} olefins (C-H) , 1200-1300 cm^{-1} Strong CS , 1400 to 1550 cm^{-1} NH , 1700 cm^{-1} Benzene ring , 2050 cm^{-1} N=C=S , 3400 free OH and at 3450 NH_2 medium .X-RD spectra of fatty acid of thiosemicarbazide of *Linum usitatissimum* (Linseed) seed oil indicate a= 8.5563 , b=7.9324 & c= 6.8432 using Hesse-Lipson procedure shows that the structure orthorhombic .The antibacterial activity of fatty acid of thiosemicarbazide of *Linum usitatissimum* (Linseed) seed oil was evaluated by diffusion method . It shows that antibacterial activity at varied levels in E.coli , S. aureus , and B. cereus bacteria .The bacteria B. cereus & E. coli was found to be more active & S.aureus was found to be less active in inhibition zone . The result calculated that the fatty acid of thiosemicarbazide of *Linum usitatissimum* (Linseed) seed oil posses good antibacterial activity.

Table I . Infrared Spectra of fatty acid thiosemicarbazide (FATSC) of *Linum usitatissimum* oil

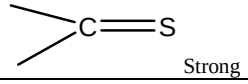
Sr. No.	Frequency Wavenumber	Expected Element
1)	750	Strong CH_2
2)	850	Other olefins C-H
3)	1050	C-OH, Strong
4)	1200 1250 1300	 Strong
5)	1400 1500 1550	---NH
6)	1700	Benzene ring
7)	1750	C=S, Stretch sulphur compounds
8)	2050	N=C=S
9)	3400	Free OH, O-H Strong
10)	3450	$-NH_2$=NH, medium

Table II. X-RD Spectra of fatty acid thiosemicarbazide(FATSC) of *Linum usitatissimum* oil

Sr. No	2Θ	hkl	Sin^2 Observed	Sin^2 Calculated	d (A^0) Observed	d (A^0) Calculated
1)	18.523	111	0.02703	0.02567	4.68412	4.89216
2)	20.165	111	0.03016	0.02998	4.32154	4.99761
3)	21.665	200	0.03170	0.03995	4.65023	4.96721
4)	24.569	210	0.04788	0.04567	3.77869	4.87693
5)	25.486	210	0.04972	0.05129	3.40563	3.87943
6)	27.499	211	0.05631	0.05876	3.24546	3.35678
7)	28.191	211	0.04898	0.05612	3.21956	3.98761
8)	28.946	211	0.04965	0.05112	3.35167	3.67832
9)	29.709	211	0.06630	0.07001	2.99785	3.87631
10)	30.459	300	0.06063	0.07681	3.00954	3.89754
11)	31.908	300	0.07322	0.07890	2.96145	3.80960
12)	32.136	300	0.07926	0.07934	2.72215	2.97658
13)	33.377	310	0.08123	0.07999	2.94567	3.85762
14)	34.747	310	0.08245	0.08114	2.99231	3.98001
15)	35.174	310	0.08290	0.08541	2.78145	3.76893
16)	35.483	310	0.09443	0.09476	2.89021	2.99780
17)	36.340	310	0.09895	0.09456	2.90614	3.91209
18)	36.842	310	0.10067	0.10011	2.42798	3.14236
19)	36.656	311	0.10371	0.13452	2.98748	3.03012

Figure-1 Infrared Spectra of fatty acid thiosemicarbazide (FATSC) of *Linum usitatissimum* oil

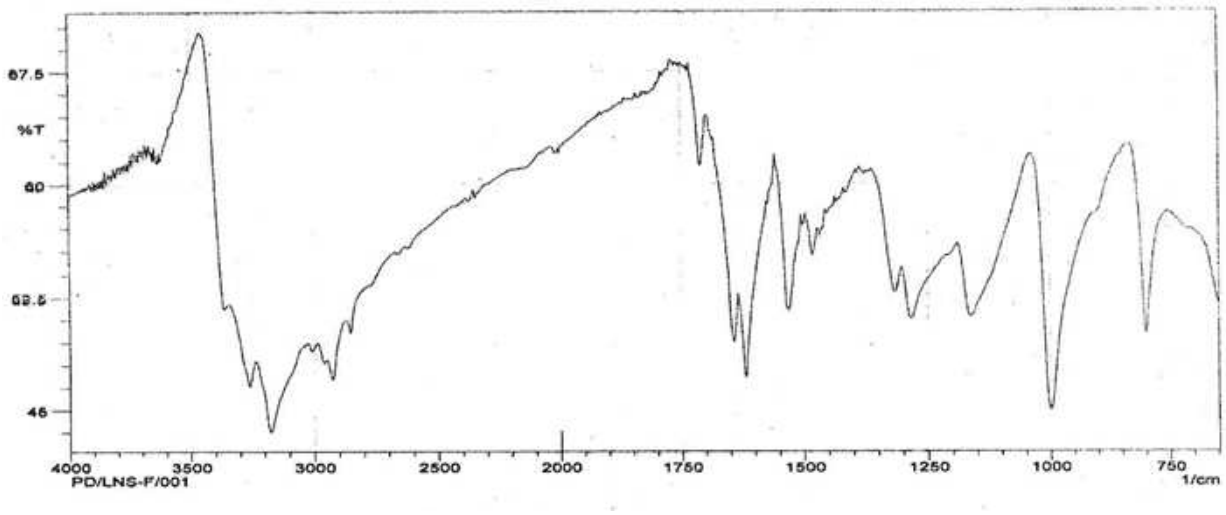


Figure 2 . X-RD Spectra of fatty acid thiosemicarbazide (FATSC) of *Linum usitatissimum* oil

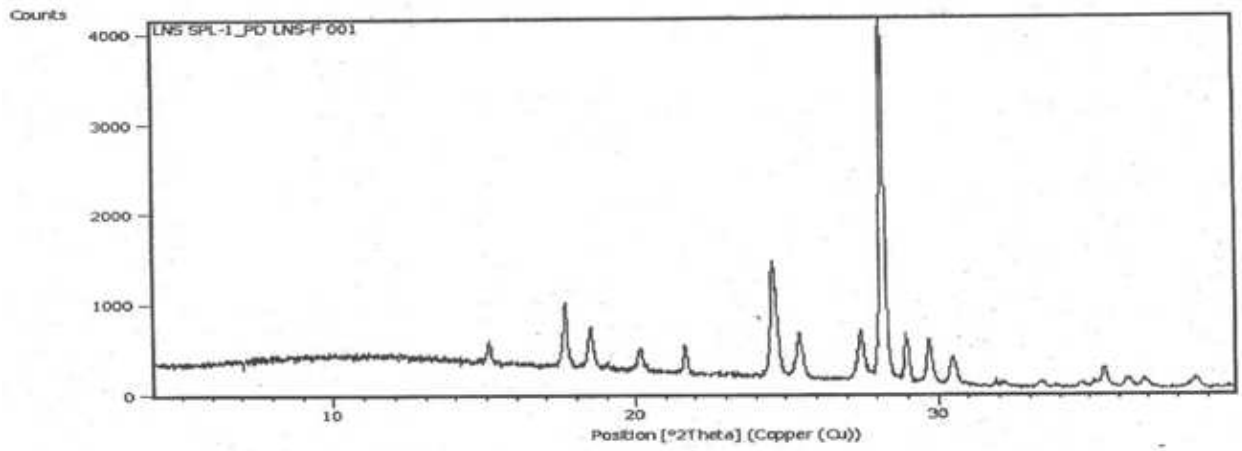


Table III. Antibacterial activity of fatty acid thiosemicarbazide (FATSC) of *Linum usitatissimum* oil

Bacteria	Reference Substance	Inhibition Zone	
		FATSC	
		150 µg / well	200 µg / well
E. coli	35.60 ± 0.53	26.34 ± 0.32	28.97 ± 0.43
S. aureus	39.10 ± 0.95	12.87 ± 0.21	25.14 ± 0.25
B. cereus	36.67 ± 0.61	31.65 ± 0.65	33.24 ± 0.54

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