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# SYNTHESIS, CHARACTERISATION & ANTIMICROBIAL ACTIVITY OF ESSENTIAL ( OMEGA-3-FATTY ACID ) THIOSEMICARBAZIDE OF LINUM USITATISSIMUM (LINSEED) SEED OIL

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## ABSTRACT

There are two subclasses of long-chain polyunsaturated fatty acids (PUFAs), omega-3-fatty acid α-Linolenic acid or ALA (18:3n-3) & omega-6-fatty acid gamma-linolenic acid or GLA (18:3n-6), . Linum usitatissimum (Linseed) seed oil used for synthesis of thiosemicarbazone derivative contains 28.05 % alpha-linolenic ( omega-3 fatty acid ) & 18 % linoleic ( omega-6 fatty acid ). Seed oil was extracted by n-hexane & fatty acid present in the seed oil is necessary for the synthesis of derivative with thiosemicarbazone. Thioemicarbazones are potent intermediates for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in the field of medicinal chemistry Omega-3 –fatty acid thiosemicarbazide of Linum usitatissimum (Linseed) seed oil was synthesized. The absorption spectra, infrared spectra and X-ray diffraction of essential ( omega-3-) fatty acid thiosemicarbazide was studied. The antibacterial activity of essential fatty acid thiosemicarbazide was studied.

**KEY WORDS**:- Linum usitatissimum (Linseed) seed oil , Thioemicarbazide , Antibacterial sample, IR, X-RD.

## INTRODUCTION

Linum usitatissimum (Linseed) is a member of the genus Linum in the family Linaceae. It is among the oldest fibre crops in the world. It is use for the production of linen dates back 5 000 years. Approximately 200 species of Linum (flaxseed or linseed) are known [1]. Flax was first brought toNorth America for its stem fiber to use in making of linen and paper. It is

an important oil seed crop. It is grown for its use as an edible oil, as a nutritional supplement, nutritional characteristics and equal numbers of short-chain omega-3 fatty acids. Linum usitatissimum (Linseed ) seed oil has various industrial and medicinal uses. It has has been under cultivation since the dawn of civilization. It contain high levels of dietary fiber as well as lignans, an abundance of micronutrients. Seed oil obtained from the seeds of Linum usitatissimum (Linseed) It has been used as a valuable compound in human nutrition. It offers high amounts of polyunsaturated fatty acids, with about 40–68% alpha-linolenic acid and 10–30% linoleic acid [2-5]. Flax may also lessen the severity of diabetes by stabilizing blood-sugar levels [6]. There is some support for the use of linseed seed as a laxative due to its dietary fibercontent [7] though excessive consumption without liquid can result in intestinal blockage [8]. Consuming large amounts of flax seed may impair the effectiveness of certain oral medications, due to its fiber content [8]. Lignans appear to be anticarcinogenic compounds [9]. The National Cancer Institute has evaluated flaxseed, along with a number of other potential food ingredients, as a component of designer foods [10]. Designer foods may be defined as those foods composed of one or more ingredients that contribute essential nutrients for health but also protect against certain diseases such as cancer and coronary heart disease Linseed oil is a rich source of linolenic acid (40-60%), an Omega 3 fatty acid which has anti-inflammatory action in the treatment of arthritis [11]. Linum usitatissimum (linseed) seed in the treatment of cancer, arthritis and cardiological diseases [12]. Linseed taken in the diet may benefit individuals with certain types of breast [13,14] and prostate cancers [15]. It may also stunt the growth of prostate tumors [15]. Scientists at American National Cancer Institute singled out flaxseed as one of six nutraceuticals for food applications [16]. Flax may also lessen the severity of diabetes by stabilizing blood-sugar levels [17]. Therapy with oral flaxseed oil capsules (1 or 2 g/day) reduces ocular surface inflammation and improves the symptoms of keratoconjunctivitissicca in Sjögren's syndrome patients [18]. A review of the literature on flaxseed yielded 13 categories for which flaxseed had been studied in humans, including for exemple constipation/laxative, attention-deficit hyperactivity disorder, hyperlipidemia, atherosclerosis/coronary artery disease and human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) [19]. Moreover, the study of Arvind (2006), explored the antioxidative properties of linseed oil in the prophylactic action against oxidative stress induced by a radiomimetic drug, cyclophosphamide . Our ethnopharmacological survey undertaken in southwest Algeria revealed that Linumusitatissimum L. has a long history of use in folk medicine as a treatment against various ailments Flax seeds contain high levels of dietary fiber and omega-3 fatty acids [20].

Thiosemicarbazide is synthetic compounds have a variety of applications [21]. These compounds have revealed wide spectrum of activities which includes anticancer [22] , anti HIV [23] , antitubercular [24] , antiviral [25] ,antitumor [26] , antiprotozoal [27], anticonvulsant [28], antidepressant [29] , antimalarial [30] , antifungal [31] , antibacterial [32] as well as parasiticidal activity against plasmodium falciparum [33] , Trypanosomacruzi [34] and and toxiplasmagondii [35].

**Collection of materials**

The dried Linum usitatissimum (Linseed) seeds were obtained from local market in Ahmednagar, Dist Ahmednagar, Maharashtra, India. They are dried in room, clean and stored in a sealed vessel wrapped with polyethylene bag at $4^0$C. Linum usitatissimum (Linseed) seed oil , Methanol, NaOH, High purity water , Sulphuric acid.The instrument used were UV spectrophotometer with wavelength range 200-450 nm. IR spectra of FAME was taken in the range of 4000$cm^{-1}$ to 600 $cm^{-1}$ on perkin Elmer 221 IR spectrophotometer using KBR pellet techniques & LCMS chromatogram.

**Extraction of oil**

After cleaning and removal of the sand and foreign material , the dried Linum usitatissimum (Linseed) seeds were ground to a fine powder using a grinde. The oil was extracted with n-hexane ( 1:4 w/v ) by continous extraction in a soxhlet apparatus for 12 hours.

**Preparation of Mixed Fatty Acids from oil**

Mixed fatty acids from Linum usitatissimum (Linseed) sseed oil were obtained by saponification method in which 100 gm oil was taken in 250 ml round bottom flask and 30 % alcoholic NaOH was added. The content were refluxed for 4 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 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.

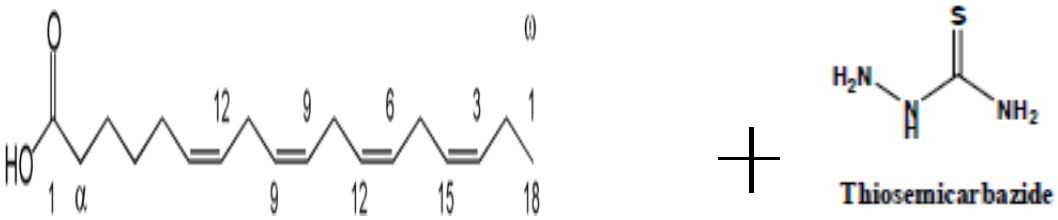
**Separation of Fatty Acid**

Fatty acids are separated by TLC on silica gel plates with hexane / diethyl ether ( 85/ 15 , v/v) as eluent. It detected after primuline spray under UV light. Spot corresponding to the respective fatty acid present .Standard solution of omega-3-fatty acid was prepared (Commercial compound ) spot on TLC obtained by fatty acid compared with the spot of

standard. Omega-3 fatty acid from mixed fatty acid is separated by micro-column filled with silica gel (3cm ) suspended in hexane ( fatty acids being dissolved in the same solvent ) .Normal fatty acids are eluted by 4 ml of hexane / diethyl ether ( 93/7, v/v ) & hydroxyl fatty acids by 4 ml of hexane / diethyl ether ( 50/50 , v/v ) the separated fatty acid omega-3-fatty acid used for the preparation of derivative of thiosemicarbazide.

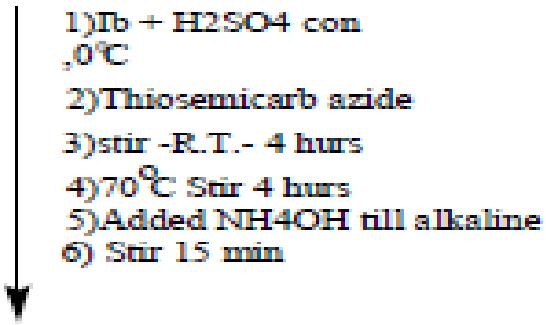
**Preparation of Essential Fatty Acid Thiosemicarbazone ( EFATSC )**

Essential fatty acid (omega-3-fatty acid ,1 gm) were dissolved in 4 ml of methanol and 1:1 $H_2SO_4$, to this solution thiosemicarbazide (4gm) in methanol was added with constant stirring at room temperature about 4 hrs and then reflux at 4 hrs added $NH_4OH$ till alkaline stir about 15 min and kept it overnight. Crystals was filtered, dried and recrystallized.




**Alpha Linolenic Acid (LNA) / Omega-3 fatty acid**

**( 6Z,9Z,12Z,15Z )-octadeca-6,9,12,15-tetraenoic acid**




Omega-3


(6*E*,9*E*,12*E*,15*E*)-1-(5-amino-1,3,4-thiadiazol-2-yl)octadeca-6,9,12,15-tetraen-1-one

**Essential ( Omega-3 ) fatty acid derivative of thiosemicarbazone**

**Absorption Spectra of EFATSC**

The absorption spectra of essential (omega-3) fatty acid thiosemicarbazone ( EFATSC ) was recorded against a blank solution shown in Fig. 1 The absorption spectra was recorded in the wavelength range 220-500 nm. EFATSC shows the absorption maximum at 395 nm shows absorption 0.199

**Infrared Spectra of EFATSC**

The infrared spectra of essential (omega-3) fatty acid thiosemicarbazide ( EFATSC ) was taken in the range of 4000 $cm^{-1}$ to 750 $cm^{-1}$ on perkin Elmer 221 IR spectrophotometer using KBR pellet techniques . The characteristic bands observed are as in Table 1. Fig. 2. Shows IR spectra of EFATSC.

**X-RD Spectra of EFATSC**

X-RD spectra of essential (omega-3) fatty acid thiosemicarbazide (EFATSC) was taken on PW 3710 diffractometer using $CuK_2$ radiation ( Y= 1.54060 ) .The X-RD diffraction of EFATSC recorded at angle 2θ from 18.523 to 36.656. The data of X-ray diffraction of EFATSC were presented in Table 2. and X-RD spectrum in Fig.3. for the determination of a, b & c Hesse-lipson procedure is used.

**Antibacterial Activity of EFATSC**

Antibacterial Activity of essential (omega-3) fatty acid thiosemicarbazide (EFATSC) of Linum usitatissimum (Linseed) seed oil was analyzed. Table 3. Well diffusion method was used for in vitro antibacterial testing Nutrient agar plates, nutrient agar slant and nutrient broth were prepared and kept for sterility testing at $37^0$C for 24 hrs. Next day pure culture of E.coli , Staphylococcus aureus and Aspergellus niger were inoculated on nutrient agar slant to obtain 24 hrs. Fresh culture of microorganisms. & kept in broth for 6 hrs. Crftriaxone was used as s standard Using stock solution 40µ / well antibacterial assay was carried out by agar well diffusion method .After 6 hrs each plate is examined Table 3,

**RESULT AND DISCUSSION**

Linum usitatissimum (Linseed) seed oil indicate a high composition of poly unsaturated fatty acid is an assest in nutrition as high content of saturated fatty acid is implicated in cardiovascular diseases. It contain fatty acid that helps to maintain healthy blood vessels. Experimentally it found that Linum usitatissimum (Linseed) seed oil is used a medicinal important. Absorption spectra of essential ( omega-3-) fatty acid thiosemicarbazide (

EFASC) of shows maximum absorption 0.199 at 395 nm. Infrared spectra of EFASC shows that at 810 $cm^{-1}$ trisubstituted alkenes, at 995 $cm^{-1}$ $RCH=CH_2$, at 1175 $cm^{-1}$ alkanes, germinal dimethyl R-O-R, at 1300 & 1330 $cm^{-1}$ S=O, Stretch, at 1445 $cm^{-1}$ $-CH_2-$, at 1550 $cm^{-1}$ nitrogen heterocycles combination C=C & C=N stretch, at 1625 $cm^{-1}$ aromatic homocyclic compounds, at 1630 $cm^{-1}$ -N=N-, stretch, at 2130 $cm^{-1}$ -N=C=N- stretch, at 2600 $cm^{-1}$ S- H, stretch, at 2860 $cm^{-1}$ alkanes $(-CH_3)-$ , at 2910 $cm^{-1}$ alkanes (-CH-), at 3150 $cm^{-1}$ bonded –NH, at 3280 $cm^{-1}$ bonded –OH, at 3350 $cm^{-1}$ N-H stretch, at 3600 $cm^{-1}$ O-H stretch. X-RD spectra of essential (omega-3-) fatty acid thiosemicarbazide (EFATSC) of Linum usitatissimum (Linseed) seed oil indicate a= 8.5563, b= 7.9324 , c= 6.8432 using Hesse-Lipson procedure shows that the structure is orthorhombic. The antibacterial activity was evaluated by diffusion method. It shows that antibacterial activity at varied level in E.coli, S. aureus & A.niger. The bacteria S.aureus, E.coli & A.niger was found to be more active in inhibition zone .The result calculated that EFATSC of Linum usitatissimum (Linseed) seed oil posses good antibacterial activity. Essential fatty acids are the polyunsaturated fatty acids .Only two fatty acids are known to be essential for human , alpha-linolenic acid (an omega-3 fatty acid ) & linoleic acid ( an omega-6 fatty acid ).Linumusitatissimum(Linseed) seed contains 35 % fats & 32 % of oil in the seed. Linumusitatissimum(Linseed) seed oil hastitre $^0C$ mp is 19-21 $^0C$ . The proximate analysis ofLinumusitatissimum(Linseed) seed oil contains 4.05 % moisture & 3.60 5 ash. It has congeding point 12.75 $^0C$, Specific gravity at 25 $^0C$ is 0.8325, refractive index at 40 $^0C$ is 1.4525 respectively. Chemical properties of linseed oil shows acid value 1.05 mg KOH/g oil. Its peroxide number is 0.98 Mev/Kg. Linumusitatissimum (Linseed) Seed oil is an excellent source of nutrition. Semicarbazones are potent intermediates for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in thefield of medicinal chemistry Omega-3 –fatty acid semicarbazide of Linumusitatissimum (Linseed) seed oil was synthesized. The absorption spectra, infrared spectra and X-ray diffraction of essential fatty acid semicarbazide was studied. The antibacterial activity of essential fatty acid semicarbazide was studied. The bacteria was found to be more active.

**Table 1..Infrared Spectra of Essential (omega-3) Fatty acid Thiosemicarbazide (FATSC) of Linum usitatissimum (Linseed) seed oil**

| Sr.No. | Frequency Wavenumber | Expected Elements |
|---|---|---|
| 1 | 810 | Trisubstituted alkenes |
| 2 | 995 | $RCH=CH_2$ |
| 3 | 1175 | Alkanes, germinal dimethyl |
| 4 | 1300<br>1330 | S=O, Stretch |
| 5 | 1485 | $-CH_2-$ |
| 6 | 1550 | Nitrogen heterocycles combination C=C & C=N , Stretch |
| 7 | 1625 | Aromatic homocyclic compound |
| 8 | 1630 | -N=N-, stretch |
| 9 | 2130 | -N=C=N-, stretch |
| 10 | 2600 | S- H, stretch |
| 11 | 2860 | Alkanes $(-CH_3)-$ |
| 12 | 2910 | Alkanes (-CH-) |
| 13 | 3150 | Bonded -NH |
| 14 | 3280 | Bonded -OH |
| 15 | 3350 | N-H ,stretch |
| 16 | 3600 | O-H Stretch |

**Table 2. X-RD Spectra of Essential (omega-3) Fatty acid Thiosemicarbazide ( EFATSC) of Linum usitatissimum (Linseed) seed 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 | 0.01037 | 0.13452 | 2.98748 | 3.03012 |

a= 8.5563 b= 7.9324 c= 6.8432

**Table 3. Antibacterial Activity of Essential (omega-3) Fatty acid Thiosemicarbazide ( EFATSC ) of Linum usitatissimum (Linseed) seed oil**

| Sr.No. | Bacteria | Reference Substance | Inhibition Zone ( EFASC ) 40 µ / well |
|---|---|---|---|
| 1 | E.coli | 40 + 2.0 | 32 + 00 |
| 2 | S.aureus | 43 + 1.0 | 38+ 0.5 |
| 3 | A.niger | 19 + 2.0 | 17 + 0.5 |

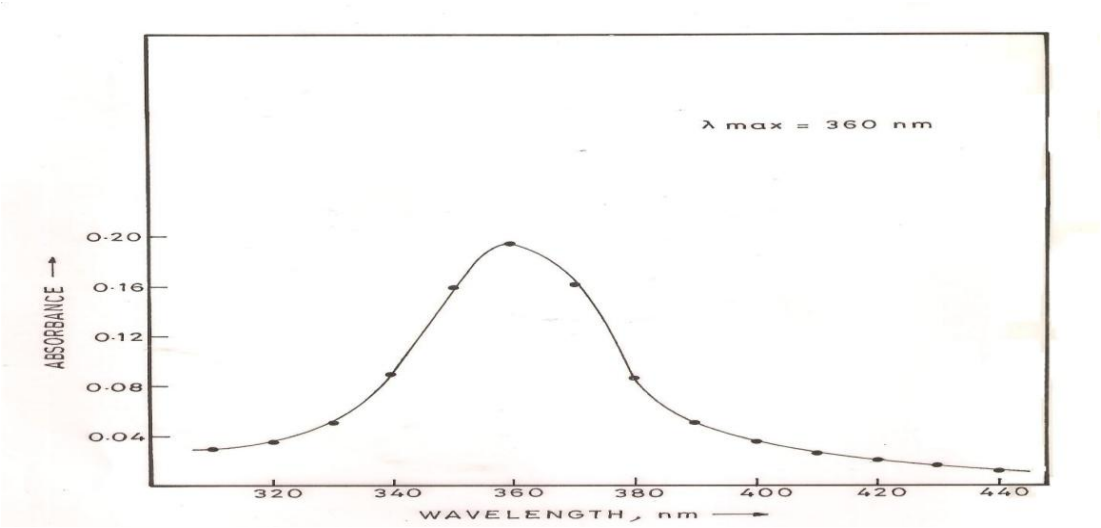



**Figure-1. Absorption Spectra of Essential Fatty acid Thiosemicarbazide ( EFATSC ) of Linum usitatissimum (Linseed)seed oil**

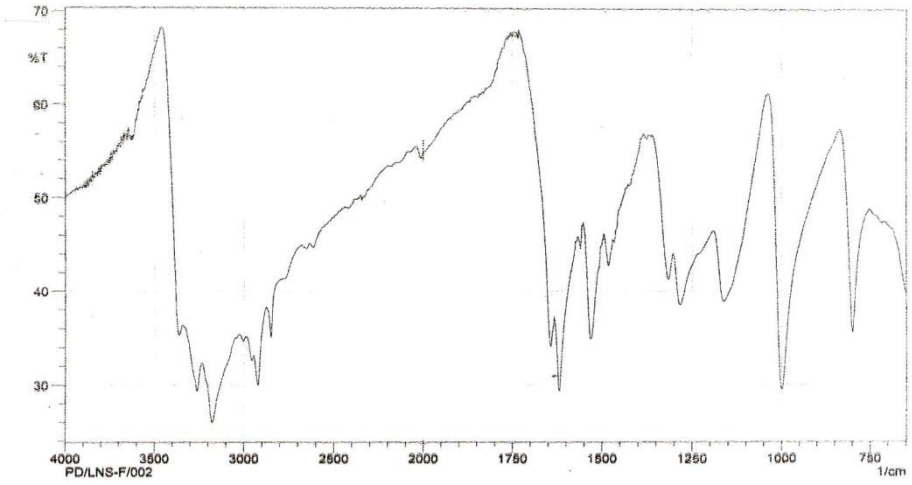



**Figure-2 . Infrared Spectra of Essential (omega-3) Fatty acid Thiosemicarbazide ( EFATSC ) of Linum usitatissimum (Linseed)seed oil**

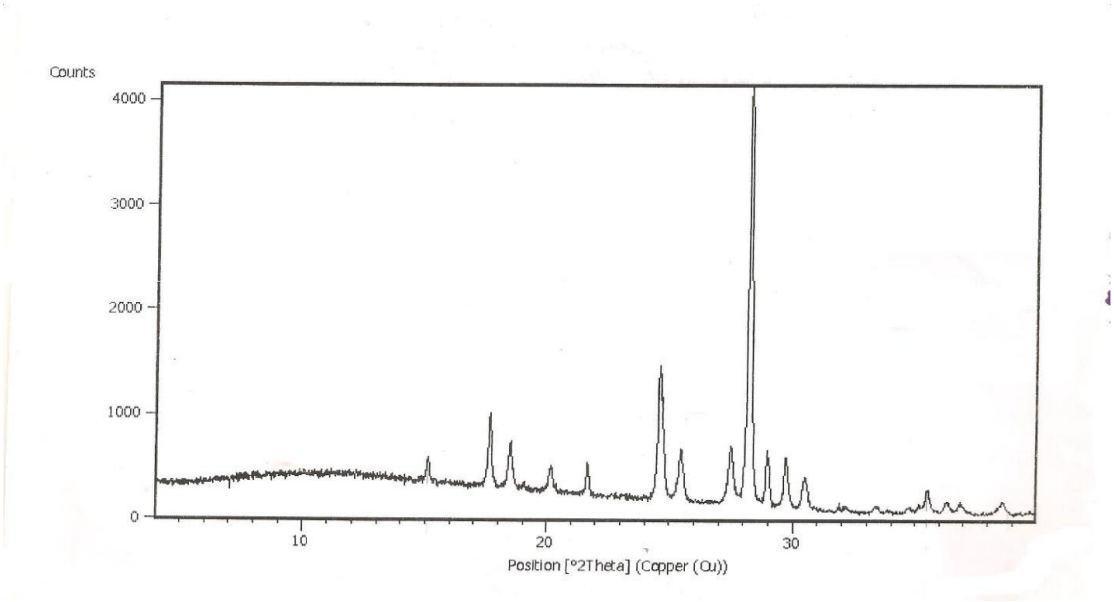



**Figure-3 . X-RD Spectra of Essential (omega-3) Fatty acid Semicarbazide ( EFATSC ) of Linum usitatissimum (Linseed)seed oil**

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