International Journal of Chemical Sciences and Applications
ISSN 0976-2590, Online ISSN 2278 – 6015
Vol 5, Issue 2, 2014, pp 46-55
http://www.bipublication.com

Synthesis, Characterisation and Antimicrobial Activity of Essential Fatty Acid of Semicarbazide

Shobha Borhade

Department of Drug Chemistry, S.M.B.S.T.College, Sangamner, Dist Ahmednagar,University of Pune, Maharashtra, INDIA-422605

[Received-07/06/2014, Accepted-21/06/2014]

ABSTRACT

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). *Linum usitatissimum* (Linseed) seed contains 35 % fats & 32 % of oil in the seed. *Linum usitatissimum* (Linseed) seed oil has titre ^{0}C mp is 19-21^{0}C . The proximate analysis of *Linum usitatissimum* (Linseed) seed oil contains 4.05 % moisture & 3.60 5 ash. It has congeding point 12.75^{0}C, Specific gravity at 25^{0}C is 0.8325, refractive index at 40^{0}C 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. *Linum usitatissimum* (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 *Linum usitatissimum* (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.

Keywords: - Linum usitatissimum (Linseed) seed oil , Semicarbazide, Antibacterial sample, IR, X-RD..

INTRODUCTION

Essential fatty acids were discovered in 1923. Essential fatty acids are the polyunsaturated fatty acids. Essential fatty acids, or EFAs, are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them [1] It plays an

important role in the transport of nutrients , helps brain development and function in children , helps support normal blood flow and transports oxygen from red blood cells to the tissue , promotes cardiovascular health in combination with a healthy diet and exercise , support healthy blood pressure , helps promote healthy metabolism , helps maintain a healthy immune system , promotes maintenance of healthy skin, hair and nails , helps fight infection , helps reduce inflammation in the body which can benefit certain inflammatory disorders like rheumatoid arthritis.

Only two fatty acids are known to be essential for human -alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid (an omega-6 fatty acid) [2]. There are two subclasses of long-chain polyunsaturated fatty acids (PUFAs), Omega -3 fatty acids - α-Linolenic acid or ALA (18:3n-3), eicosapentaenoic acid or EPA (20:5n-3), docosahexaenoic acid or DHA (22:6n-3), Omega-6 fatty acids:- gamma-linolenic acid or GLA (18:3n-6), Linoleic Acid (LA) or LA (18:2n-6), dihomo-gamma-linolenic acid or DGLA (20:3n-6). The importance of omega-3 fatty acids for physical well-being has been recognised for several decades [3]. Omega-3-fatty acids have anti-inflammatory, antithrombotic antiarrhythmic and hypolipidaemic effects [3] ,becauseof this, these fatty acids are beneficial in the prevention and treatment of physical illnesses ranging from coronary heart disease [4] to rheumatoid arthritis.[3],homo sapiens evolved in an omega-3-rich nutritional environment [5]. More recently, there has been increasing evidence that omega-3 fatty acids are important notonly for physical health but also for brain develop-ment and function [7-9]. As a result, there has bee nincreasing interest in the use of omega-3 fatty acids for the treatment of mental health problems. Omega-3 fatty acids have important effects on brain function. DHA is a major structural compound. Eicosapentaenoic acid (EPA) and docosahexae- nent of phospholipid in neuronal cell membranes [10-11]. The first suggestion that omega-3 fatty acid smight be helpful for the treatment of mental disorder was made by Rudin [12-13]. who suggested that mentalhealth problems were caused by omega-3 fatty acid deficiency and reported successful treatment of a number of patients with flax oil, which is a richsource of ALA.. The modern resurgence of interest in the therapeutic benefits of omega-3 fatty acids was the direct result of observations that levels of omega-3 fatty acids are reduced in the cell membranes of erythrocytes of patients with schizophrenia and depression [14-16]. The first studies with fish oil derivatives were carried out in schizophrenic patients, and this was closely followed by studies in patients with mood disorders. Studies in patients with depressionwere further supported by epidemiological evidence showing that international variations in the prevalence of depression correlated closely with fish consumption in the national diet [17]. It is important to consume an ideal ratio of Omega-6 to Omega-3. The ideal ratio is between 4:1 and 3:1 Omega-6 to Omega-3 but the average person has an intake somewhere between 10:1 to 25:1.

Omega-3 Content of Natural Oils -Linseed 53-62%, Hemp seed 53%, Pumpkin Seed oil 46%, Canola 11%, Walnut 10%, Wheat germ 7% Soybean [18-19].

Linum usitatissimum (Linseed) is a multi-purpose crop. Its' seeds containing about 36 to 40 % of oil, have long been used in human and animal diets and in industry as a source of oil and as the basic component or additive of various paints or polymers. Recently there has been a growing interest in the probiotic properties of Linum usitatissimum (Linseed) and in its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders [20-22]. The beneficial effects are mostly due to linseed lipids. Linum usitatissimum (Linseed) seed oil is the richest plant source of linoleic (omega-6) and linolenic (omega-3)

polyunsaturated fatty acids (PUFA), which are essential for humans since they cannot be synthesized in the organism and must be ingested in food. Linum usitatissimum (Linseed) seed oil is qualitatively different from the more common vegetable oils with high PUFA proportions, such as soya oil, sunflower oil, rape oil, olive oil, etc. Linum usitatissimum (Linseed) seed oil is a rich source of the following unsaturated fatty acids: oleic (C18,16–24 %),linoleic (C18, 18–24 %), and linolenic acid (C18, 36–50 %) [23] and it has a relatively low glucosinolate content [24]. The protein and fiber content in the seed are also important nutritional parameters: the crude protein content in the seed ranges from 25 % to 45 %, while the crude fiber content is about 10 % [25] .The results of the analyses of Linum usitatissimum (Linseed) seed oil have been reported elsewhere [26,27]. Unsaponifiable lipid constituents of seed oils naturally contain hydrocarbons, terpene alcohols, sterols, tocopherols and other phenolic compounds which may act as oxidation inhibitors under a range of conditions [28] .The effectiveness of lipid unsaponifiable matters in retarding oil deterioration has beendemonstrated by many investigators [29-30] In Linum usitatissimum (Linseed) seed grains, lipids are protected against oxidation by various mechanisms, for example, the presence of antioxidants such as lignans, phenols, tocopherols -(vitamin E) and flavonoids [31- 32] .In addition to preventing fat rancidity, these antioxidants could increase commercial value of food products and have beneficial effects on human health. When consumed together with essential unsaturated fatty acids, they can reduce the risk of various diseases [33]. The antioxidant ability of phenols, tocopherols (vitamin E) and flavonoids is related to the presence of OH groups which may directly bind to free radicals and chelate metals [34]. *Linum usitatissimum* (Linseed) is a nutritional supplement with high concentrations of (n-3) fatty acids and lignans that have anti-inflammatory and antioxidant properties.

Semicarbazides are the raw material of semicarbazones, have been known to have biological activity against many of the most common species of bacteria [35-37] . Semicarbazones constitute one of the most important class of oxygen and nitrogen donar ligands [38-43].Semicarbazone, themselves are of much interest due to a wide spectrum of antibacterial activities [44]. Recently some workers had reviewed the bioactivity of semicarbazones and they have exhibited anticonvulsant [45,46], antitubercular [47], analgesic and anti-inflammatory activity [48], antimicrobial [49], pesticide [50], herbicide [51] and hypnotic [52].

MATERIALS AND METHODS

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 4^0C.

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. The solvent was evaporated at 40^0C in rotavapour. The extracted

oil was stored in sealed and dark bottles. Their physic-chemical analysis was done by standard BIS methods. 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

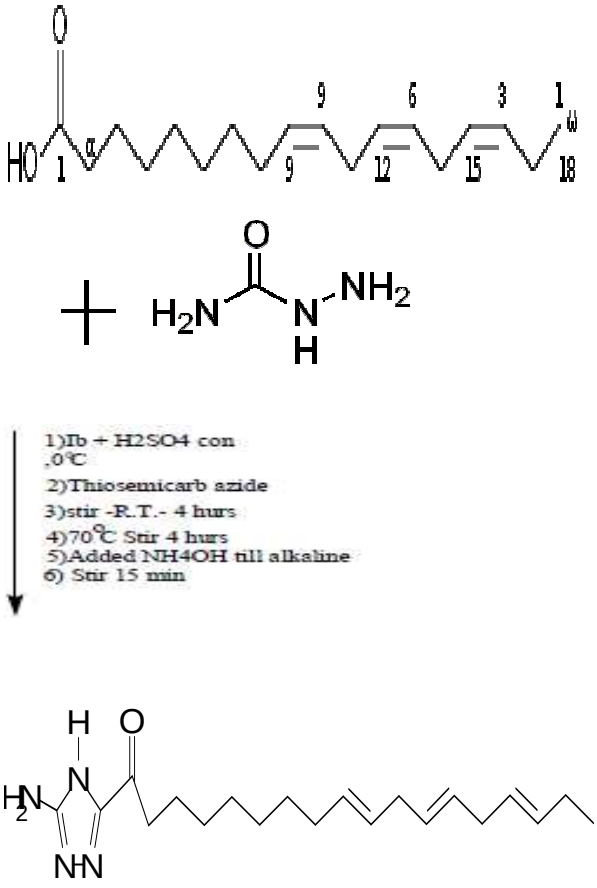
Mixed fatty acids from *Linum usitatissimum* (Linseed) seed oil were obtained by saponification method in which 100 gm oil was taken in 250 ml round bottom flask and 30 % alcoholic NaOH was added. The content were refluxed for 3 hrs. on stirring water bath. At the end of the reaction, the excess alcohol was distilled off and soap was dissolved in hot water. Then 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-3-6--fatty acid was prepared (Commercial compound) spot on TLC obtained by fatty acid compared with the spot of standard. Omega-3-6-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-3-6-fatty acid used for the preparation of derivative of semicarbazide.

Preparation of Essential Fatty Acid Semicarbazide (EFASC)

Essential fatty acid (omega-3-6-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.



Essential fatty acid semicarbazide derivative (EFASC)

Absorption Spectra of EFASC

The absorption spectra of essential fatty acid semicarbazide (EFASC) was recorded against a blank solution shown in Fig. 1 The absorption spectra was recorded in the wavelength range 320-520 nm.EFASC shows the absorption maximum at 370 nm shows absorption 3.182.

Infrared Spectra of EFASC

The infrared spectra of essential fatty acid

semicarbazide (EFASC) was taken in the range of 4000cm^{-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 EFASC.

X-RD Spectra of EFASC

X-RD spectra of essential fatty acid semicarbazide (EFASC) was takenon PW 3710 diffractometer using CuK_2 radiation (Y= 1.54060) .The X-RD diffraction of EFASC recorded at angle 2θ from 10.9872 to 38.6561. The data of X-ray diffraction of EFASC were presented in Table 2. And X-RD spectrum in Fig.3. for the determination of a,b & c Hesse-lipson procedure is used [53].

Antibacterial Activity of EFASC

Antibacterial Activity of essential fatty acid semicarbazide (EFASC) 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 37^0C 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 wasused as s standard Using stock solution 40µ / well antibacterial assay was carried out by agar well diffusion method [54-56]. After 6 hrs each plate is examined Table 3, Fig 4.

RESULTS AND DISCUSSION

Linum usitatissimum (Linseed) seed is reddish colour & its oil is yellow in colour with pleasant nutty taste, paint like odour. Its acid value & peroxide number is 1.05 mg KOH/g of oil , 0.98 Meg/Kg . Iodine value is 163.5 g/100 g of oil it indicate a high composition of poly unsaturated fatty acid ia 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 fatty acid semicarbazide (EFASC) of *Linum usitatissimum* (Linseed) seed oil shows maximum absorption 3.182 at 370 nm.Infrered spectra of EFASC shows that at 800 cm^{-1} ring containing three adjacent H atoms, at 960 cm^{-1} disubstituted alkenes ($R_1CH=CHR_2$), at 1000 cm^{-1} ($R_1CH=CHR_2$), at 1150 cm^{-1} methylene ester R-$COOCH_3$, at 1225 cm^{-1} -C=O , stretch, at 1300 cm^{-1} solid fatty acid (CH_2 Vib.), at 1350 cm^{-1} O-H, stretch, at 1425 cm^{-1} & 1600 cm^{-1} O=C-O, at 1680 cm^{-1} cyclic C=N, azomethane, at 2550 cm^{-1} organosulphur S-H stretch, 2570 cm^{-1} & 2580 cm^{-1} R-C=N=N, at 3000 cm^{-1}& 3050 cm^{-1} C-H Stretch. X-RD spectra of essential fatty acid semicarbazide (EFASC) of *Linum usitatissimum* (Linseed) seed oil indicate a= 6.9765, b= 5.3269 & c= 7.4727 using Hesse-Lipson procedure shows that the structure is orthorhombic. The antibacterial activity was evaluated by diffusion method. It shows thatantibacterial activity at varied level in E.coli, S. aureus & A.niger. The bacteria S.aureus was found to be more active in inhibition zone than E coli & A.niger. The result calculated that tha EFASC of *Linum usitatissimum* (Linseed) seed oil posses good antibacterial activity.

REFERENCES

1. Groff J L, Gropper S S, Hunt S M.,(1995), Advanced Nutrition and Human Metabolism. New York, West Publishing Company.
2. McGuire G l, (2004), Pharmaceutical classes, TherapeuticAgents, Area of application. (Miscellaneous drug Related Technology), 4, 344, 2046.
3. Simopoulos A P., Essential fatty acids in health and chronic condisease. Am J Clin Nutr (1999), 70 ,3, 560S-9S.

4. Hu F B, Willet W C., (2002), Optimal diets for prevention of coronaryheart disease. JAMA 288, 2569-78.
5. Cleland L G, James M J, Proudman S M. (2003), The role of fish oils inthe treatment of rheumatoid Arthritis, Drugs 63 (9), 845-853.
6. Crawford M A, Bloom M, Cunnane S, et al.(2001), Docosahexaenoicacid and cerebral evolution. World Rev Nutr Diet 88,6-17.
7. Uauy R, Peirano P, Hoffman D, et al. (1996),Role of essential fattyacids in the function of the developing nervous system. Lipids 31, 167-76.
8. Wainwright P E. (2002), Dietary essential fatty acids and brain function: a developmental perspective on mechanisms. Proc Nutr Soc, 61, 61-69.
9. Salem J N, Litman B, Kim HY, et al. (2001), Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids, 36, 945-59.
10. Burdge G. (2004), Alpha-linolenic acid metabolism in men and women nutritional and biological implications. Curr Opin Clin Nutr Metab Care 7, 137-144.
11. Horrobin D F, Manku M S, Hillman H, et al. (1991), Fatty acid levels in the brains of schizophrenic and normal controls. Biol Psychiatry , 30, 795-805.
12. Fenton W S, Hibbeln J, Knable M. (2000), Essential fatty acids, lipid membrane abnormalities and the diagnosis and treatment of schizophrenia. Biol Psychiatry , 47, 8-21.
13. Rudin D O. (1981), The major psychosis and neuroses as omega-3 essential fatty acid deficiency Syndrome substrate pellagra. Biol Psychiatry ,16, 837-850.
14. Peet M, Laugharne J, Rangarajan N, et al.(1995), Depleted red cell membrane essential fatty acids in drug-treated schizophrenia patients. J Psychiatr Res ., 29, 227-232.
15. Yao J K, Van Kammen D P, Welker J A. (1994), Red blood cell membrane dynamics in schizophrenia. II, fatty acid composition. Schizophr Res .,13, 217-226.
16. Glen A L, Glen E M, Horrobin D F, et al. (1994),A red cell membrane abnormality in a subgroup of schizophrenic patients, evidence for two diseases. Schizophr Res ., 12, 53-61.
17. Hibbeln J R. (1998), Fish consumption and major depression [letter]. Lancet, 351, 1213.
18. Hunter J E. (1990), n-3 Fatty acids from vegetable oils. Am J Clin Nutr.,51, 809-814.
19. Mantzioris E, James M J, Gibson R A, Cleland L G. (1994), Dietary substitution with an alphalinolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. Am J Clin Nutr.,59,1304-1309.
20. Huang S D Milles, (1996), Gamma-linolenic acid Metabolism and its roles in nutrition and medicine. Champaign, IL, AOCS Press.
21. Huang S A. Ziboh (2001), Gamma-linolenic acid: Recent advances in biotechnology and clinical applications. Champaign, IL, AOCS Press.
22. Flachowsky G T, Langbein H, Böhme A, Schneide K, Aulrich (1997), Effect of false flax expeller combined with short-term vitamin E supplementation in pigs feeding on the fatty acid pattern, vitamin E concentration and oxidative stability of various tissues, J. Anim. Physiol. Anim. Nutr., 78, 187–195
23. Schuster A W, Friedt (1998), Glucosinolate content and composition as parameters of quality of Camelinaseed. Ind. Crops Prod., 7, 297–302.
24. Korsrud G M, Keith J, Bell (1978), A comparison of the nutritional value of crambe and camelina seed meals with egg and casein. Can. J. Anim. Sci., 58, 493–499
25. Fofana B S, Duguid S, Cloutier (2004), Cloning of fatty acid biosynthetic genes β-ketoacyl CoA synthase, fatty acid elongase, stearoyl-ACP desaturase, and fatty acid desaturase and analysis of expression in the early developmental stages of flax (Linum usitatissimum L.) seeds, Plant Science, 166, 1487–1496.
26. Lukaszewicz M J, Szopa A, Krasowska (2004),Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidant, Food Chemistry, 88, 225– 231.
27. Bosku D I,Morton (1976), Effect of plant sterols on the rate of deterioration of heated oils. J. Sci. Food Agric., 27, 928-932
28. Rady A, Awatif I, (1991), Improvement of oils stability.Fourth Arab conference of Food Science and Technology,Cairo, Egypt.Ricciarelli R J, Zingg A , Azzi (2001),Vitamin E protective role of a Janus molecule. FASEB J., 15, 2314–2325.
29. Awatif I M, Khalil H, Badawy (1996), Effect of natural and synthetic antioxidants on the stability

of virgin olive oil, Annals Agric. Sci., Ain Shams Univ., Cairo, 41(l), 269-280.
30. Kitts D Y,Yuan A, Wijewickreme L, Thompson (1999), Antioxidant activity of the flaxseed lignan secoisolariciresinoldiglycoside and its mammalian lignan metobolitesenterodiol and enterolactone, Molecular Cell Biochemistry, 202,91–100
31. Ahmed M J, Daun R, Przybylski (2005), FT-IR based methodology for quantitation of total tocopherols, tocotrienols and plastochromanol-8 in vegetable oils, Journal of Food Composition and Analysis, 18, 359–364.
32. Romieu I C, Trenga (2001), Diet and obstructive lung diseases. Epidemiologic Reviews, 23, 268–287.
33. Halliwell B J, Gutteridge M (1999), Free radicals in biology and medicine (3Ed.), Oxford University Press. Huang S D, Milles (1996), Gamma-linolenic acid Metabolism and its roles in nutrition and medicine. Champaign, IL, AOCS Press.
34. Dogan H N, Duran A, Yemni E, (1999), Drug Metab. Drug Interact, 15, 187.
35. Hulya P, Ipek Y, Uiuk A, Feth S M, Ningur N, (1993), J. Fac. Pharm., 10,117.
36. Cotti L, (1940), Biochem. Terap. Sper 27, 366.
37. Vogel A I, (1964),A Text Book of Quantitative Inorganic Analysis, 3rd Edn., ELBSLongmans,Green & Co. Ltd., London.
38. Prasad R N and Jindal M,(1989), J Indian Chem Soc., 66, 188.
39. Prasad R N, Jindal M, Jain M, Varshney A and Chand P, (1990), J Indian Chem Soc., 67, 91.
40. Prasad R N, Jindal M and Jain M, (1990), J Indian Chem Soc.,67, 874.
41. Sulekha Chandra, Krishna Sharma K,(1984),Transition Met Chem., 9,1-3.
42. Chandra S, Pandeya K B and Singh R B, (1977), J Inorg Nucl Chem., 39, 2079.
43. Pandeya S N, Dimmock J R,(1993), Pharmazie,48, 659.
44. Pandeya S N, Misra V,Singh P N, Rupainwar D C, (1998), Pharmacol., 37,17.
45. Pandeya S N, Yogeeswari P, Stables J P, (2000), Eur. J. Chem, 35, 879.
46. Sriram D,Yogeeswari P, Thirumurugan R S, (2004), Bioorg. Med. Chem. Lett, 14, 3923.
47. Singh H P,Chauhan C S,Pandeya S N,Sharma C S,Srivastava B,Singhal M (2010), Design, Synthesis, Analgesic and Anti-Inflammatory Activity of Some novel Chalcone semicarbazone derivatives, Der Pharmacia Lettre, 2 , 460-462.
48. Lever A B (1995), Inorg. Chem., 4,763.
49. Anderson R J, Cloudsdale I S, Lamoreaux R J, Schaefer K and Harr J.(2000), US Patent, 6110,869.
50. Copping L G, Kerry J C, Watkins T I, Wllis R J and Bryan H. (1983), US Patent, 4394: 387.
51. Pandeya S N, Aggarwal N and Jain J S. (1999), Pharmazie, 54: 4.
52. Azaroff L V, Burger M J (1958), The powder method-Analytical method for indexing powder photographs ,MC Graw Hill Book Co.inc.,N Y 83.
53. Hansen K, Nyman U, Smit U W, Adersen A, Gudiksen L, Rajaselharan S, Pushpangadan P (1995), Journal of Ethnopharmacology, 48 (1), 43-45.
54. Cohen J H et.al. (2002), Journal of National Cancer Institute ,92,61-68.
55. Pascual C, Gonzalea R, Torricella R G (1994), J. Ethnopharmacol, 41, 9-13.

Table 1.. Infrared Spectra of Essential Fatty acid Semicarbazide (EFASC) of *Linum usitatissimum* (Linseed) seed oil

Sr.No.	Frequency Wavenumber	Expected Elements
1)	800	Ring containing three adjacent H atoms
2)	960	Disubstituted alkenes ($R_1CH=CHR_2$)
3)	1000	($R_1CH=CHR_2$)
4)	1150	Methylene ester R-$COOCH_3$
5)	1225	-C=O , Stretch

6)	1300	Solid fatty acid (CH_2 Vib.)
7)	1350	O-H, Stretch
8)	1425 & 1600	O=C-O
9)	1680	Cyclic C=N, azomethane
10)	2550	Organosulphur S-H Stretch
11)	2570 & 2580	R-C=N=N
12)	3000 & 3050	C-H Stretch

Table 2. X-RD Spectra of Essential Fatty acid Semicarbazide (EFASC) of *Linum usitatissimum* (Linseed) seed oil

Sr.No.	**2θ**	**hkl**	**Sin^2 θ Observed**	**Sin^2 θ Calculated**	**d(A^0) Observed**	**d(A^0) Calculated**
1	10.9872	100	0.0787	0.0932	4.9321	4.7562
2	13.8725	100	0.0935	0.0147	4.8987	4.6845
3	14.0134	100	0.1432	0.1479	4.8549	4.5634
4	14.9976	100	0.1227	0.1236	4.7061	4.5999
5	16.2419	100	0.1416	0.1595	4.4020	4.3879
6	16,9987	100	0.1317	0.1399	4.3217	4.1769
7	18.0023	110	0.0946	0.0977	4.2120	4.1564
8	19.1214	110	0.1889	0.1973	4.2063	4.0923
9	20.6734	110	0.1873	0.1887	4.1199	3.9847
10	21.5763	110	0.1102	0.1209	4.1031	3.8546
11	23.9879	111	0.2103	0.2035	4.0902	3.7473
12	25.3479	111	0.1574	0.1635	3.9765	3.5792
13	26.5039	111	0.2834	0.2879	3.7314	3.3528
14	27.9897	111	0.2204	0.2321	3.6205	3.1653
15	28.7984	111	0.1534	0.1699	3.3497	3.1002
16	30.5263	200	0.1873	0.1932	3.2531	2.9564
17	31.7243	200	0.2134	0.2225	2.9890	2.8463
18	32.7012	220	0.1631	0.1712	2.7356	2.6899
19	32.9743	220	0.1536	0.1631	2.7389	2.5378
20	34.1213	220	0.2834	0.2912	2.6104	2.3298
21	34.4329	220	0.1732	0.1777	2.5994	2.1218
22	36.3024	220	0.1417	0.1514	2.3621	1.9976
23	38.6561	220	0.1260	0.1331	1.9856	1.5643

Table 3. Antibacterial Activity of Essential Fatty acid Semicarbazide (EFASC) of *Linum usitatissimum* (Linseed) seed oil

Sr.No.	Bacteria	Reference Substance	Inhibition Zone (EFASC) 40 µ / well
1	E.coli	40 + 2.0	15 + 00
2	S.aureus	43 + 1.0	17 + 0.5
3	A.niger	19 + 2.0	11 + 0.5

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

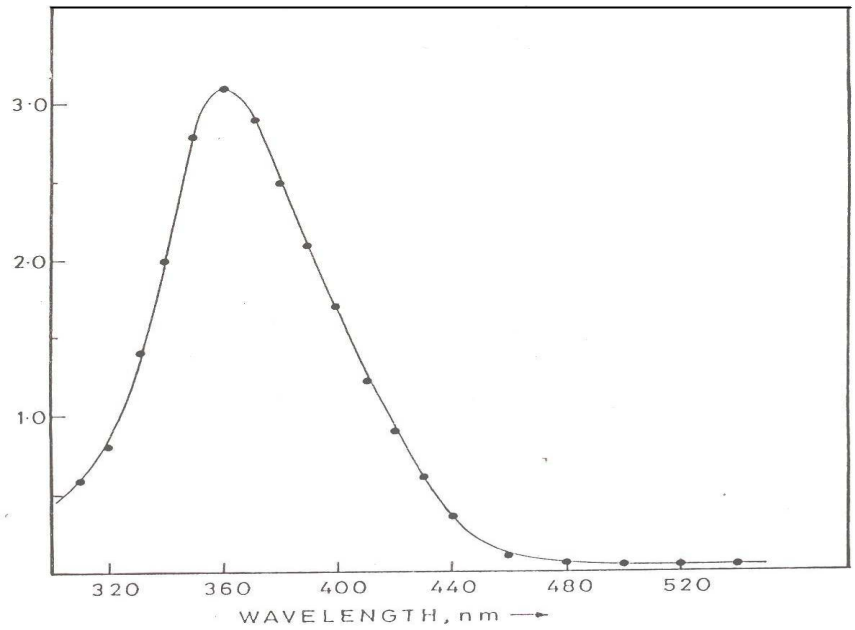


Figure-2 . Infrared Spectra of Essential Fatty acid Semicarbazide (EFASC) of *Linum usitatissimum* (Linseed) seed oil

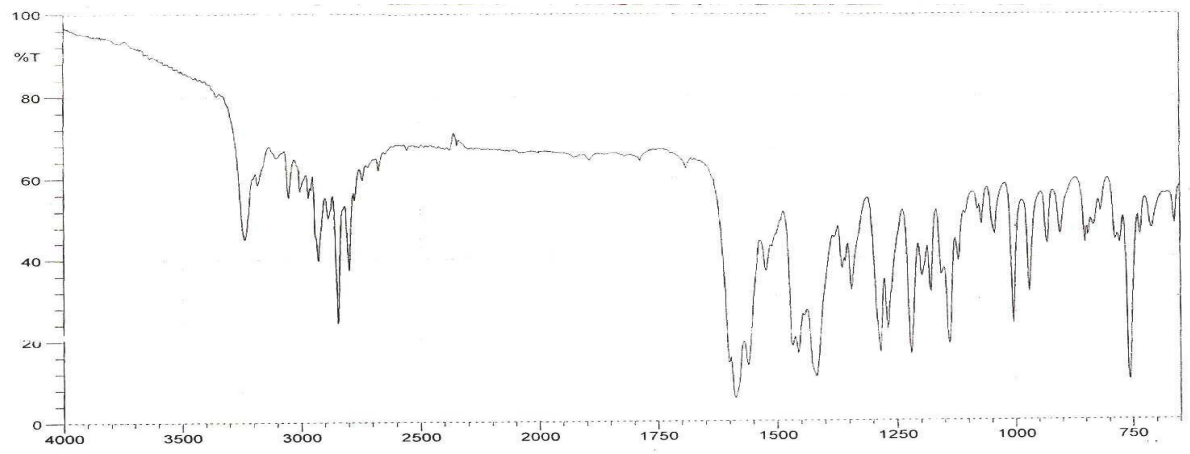


Figure-3 . X-RD Spectra of Essential Fatty acid Semicarbazide (EFASC) of *Linum usitatissimum* (Linseed) seed oil

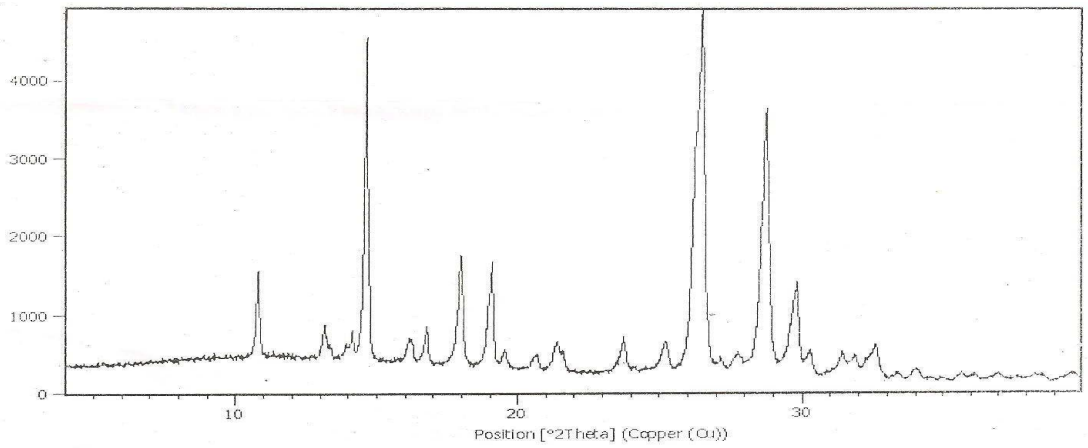
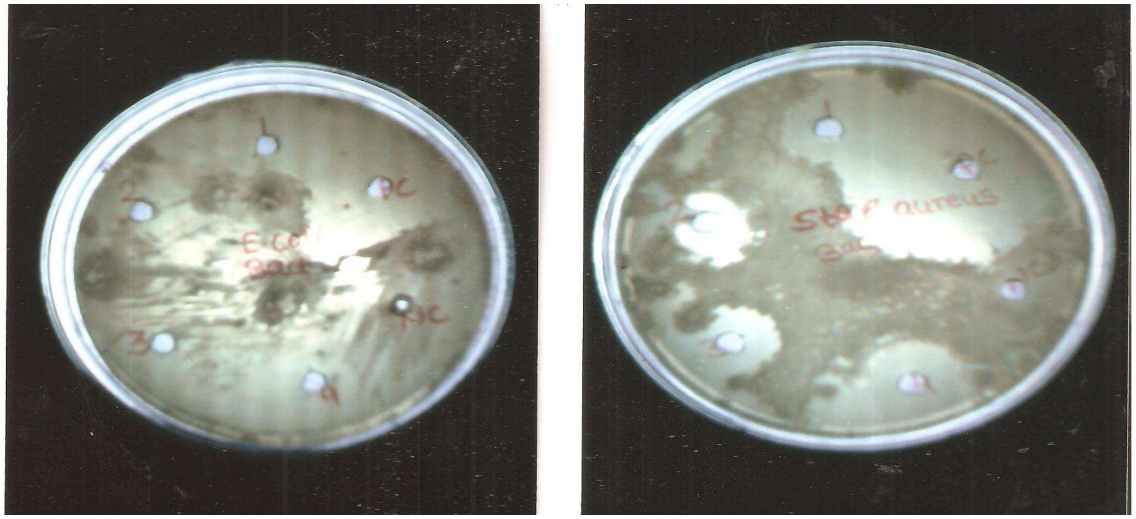


Figure-4. Antibacterial Activity of Essential Fatty acid Semicarbazide (EFASC) of *Linum usitatissimum* (Linseed) seed oil



Sample No. 4