Study of Thiosemicarbazone Derivative of Essential Fatty Acid

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Abstract

Essential fatty acids results in numerous health benefits. 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).The importance of omega-3 fatty acids for physical well-being has been recognised for several decades . Omega-3 fatty acids have anti-inflammatory, antithrombotic, antiarrhythmic and hypolipidaemic effects. Cannabis sativa (Hemp) is an angiosperm belonging to the cannabaceae family and cannabis genus. Hemp seed has unique nutritional properties and has commonly been claimed to be one of the most nutritionally complete foods and is truly one of nature's superfoods. Hemp seed contain oil 32.21 %, moisture 03.07 % , protein 23.90 % , specific gravity (25 0C) 0.8927, refractive Index at 40 0C is 1.4570, acid value 2.15 mg KOH / g of oil , saponification number 190,2 mg KOH / g of oil . Thiosemicarbazides and thiosemicarbazones as an important class of synthetic compounds, have a variety of applications . Omega-3 –fatty acid thiosemicarbazide of Cannabis sativa (Hemp) seed oil was synthesized. The absorption spectra, infrared spectra and X-ray diffraction of essential fatty acid thiosemicarbazide was studied. The antibacterial activity of essential fatty acid thiosemicarbazide was studied. The bacteria S.aureus was found to be more active

Keywords: Cannabis sativa (Hemp) seed oil , Thiosemicarbazide, Antibacterial sample, IR, X-RD..

Introduction

Modern diet is very deficient in vital nutrients. Increase in incidence and severity of many degenerative diseases including cardiovascular diseases, dibetes, cancer, arthritis, mental disorders etc. are attributed to the deficiency and more particularly to the omega-6 to omega-3 inbalance. 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) (Demain M J 2008). There are two subclass 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).

Essential fatty acids results in numerous health benefits. Documented benefits include prevention of atherosclerosis, reduced incidence of heart disease and stroke, and relief from the symptoms associated with ulcerative colitis, menstrual pain, and joint pain (Linscheer 1994, Barnard N 1994, New data 2003). Omega-3 fatty acid levels have also been associated with decreased breast cancer risk (Thiebaut et al 2009, Yee LD et al 2010). Essential fatty acid plays an important role in the transport of nutrients, It helps brain development and function in children.It promotes cardiovascular health in combination with a healthy diet and exercise, supports healthy blood pressure, helps promote healthy metabolism, helps reduce inflammation in the body which can benefit certain inflammatory disorders like rheumatoid arthritis. The importance of omega-3 fatty acids for physical well-being has been recognised for several decades (Simopoulos A P 1999). Omega-3fatty acids have anti-inflammatory, antithrombotic antiarrhythmic and hypolipidaemic effects (Simopoulos A P 1999). Because of this, these fatty acids are beneficial in the prevention and treatment of physical illnesses ranging from coronary heart disease (Hu FB, 2002) to rheumatoid arthritis (Cleland LG 2003). Homo sapiens evolved in an omega-3-rich nutritional environment (Crawford MA 2001). More recently, there has been increasing evidence that omega-3 fatty acids are important not only for physical health but also for brain development and function (Uauy R 1996, Wainwright P E 2002, Salem 2001) As a result, there has been increasing interest in the use of omega-3 fatty acids for the treatment of mental health problems (Burdge G 2004) . Omega-3 fatty acids might be helpful for the treatment of mental disorder was made by Rudin, (Fenton W S 2004, Rudin DO 1981). Omega-3-fatty acids are known to have membrane enhancing capabilities in brain cells (Manson JE et al 1999, Newcomer LM 2001, Iso H 2001). Cannabis sativa (Hemp) in addition its nutritional value, has demonstrated positive health benefits (Guil-Gurrero J L 2007, Schweb U 2006). Effects of hemp seed and flaxseed oils on the profile of serum lipids, serum total and lipoprotein lipid concentrations and haemostatic. and has traditionally been consumed in folk and folk medicinal preparations, employed as bird and poultry feed, or as a functional food (Oomah B D 2002). The interest in hemp for cosmetic products, particularly skin care, is due to the high content of favorable unsaturated fatty acids in seed of Cannabis sativa (Hemp) , particularly linoleic, α-linolenic, and γ-linolenic acids, which influence several important cell membrane functions (KrezelIzabella 1998). Therefore, the full characterization of hemp seed oils of

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diverse origins still appears to be a sound research priority to obtain a reliable data on this valuable crop.

Cannabis sativa (Hemp) is an angiosperm belonging to the cannabaceae family and cannabis genus. Hemp seed is one of the best source of essential fatty acids with perfect 3:1 ratio of omega-3-linolenic acid and omega-6-linoleic acid, good for strengthening the immune system. It is also a good source of gamma linoleic acid . The high content of omega-6 & omega-3 fatty acids and the relatively high phytosterol content of hemp make them beneficial to cardiovascular health polyunsaturated for saturated fats can reduce the risk of sudden cardiac arrest and fatal cardiac arrhythmia as well as reducing blood cholesterol levels and decreasing the cellular proliferation associated with atheroscierosis. Cannabis sativa (Hemp) seed oil is valued primarily for its nutritional properties as well as for the health benefits associated with it. Cannabis sativa (Hemp) seed has unique nutritional properties and has commonly been claimed to be one of the most nutritionally complete foods and is truly one of nature's super foods (Osburn L 1992, Deferne J L 1996). With a complete source of all essential amino and fatty acids, hemp seed oil is a complete nutritional source. In addition, constituents exist within the oil that have been shown to exhibit pharmacological activity (Erasmus U 1999). Cannabis sativa (Hemp) seed oil contains linoleic acid (LA) and α -linolenic acid(LNA) as its major omega-6 and omega-3 polyunsaturated fatty acids (PUFA) respectively. These fatty acids comprise the most desirable contents of the oil, especially due to the ratios in which they exist. The 3:1 ratio of LA to LNA is alleged to be optimal for nutrition (Xiaozhai L 1995). The additional presence of gamma-linolenic acid (GLA) in hemp seed oil ultimately makes its nutritional value superior to most comparable seed oils. The myriad of benefits reported to be attributable to omega-3 PUFA include anticancer, anti-inflammatory, and anti-thromboticproperties. In addition, dietary omega-3 PUFA help to increase general metabolic rates and promote the burning of fat (Oh K et al 1995, Simopoulos AP 1994). Hemp oil is one of the few oils that contains measurable levels of gamma-linolenic acid, an omega-6 fatty acid that is synthesized in healthy persons from linoleic acid. Hempseed, in addition its nutritional value, has demonstrated positive health benefits (Guil-Gurrero J L 2007 , Schweb U 2006 . Unsaturated fatty acids in seed of hemp, particularly linoleic, α-lino-lenic, and γ-linolenic acids, which influence several important cell membrane functions (KrezelIzabella 1998).

Thiosemicarbazides and thiosemicarbazones as an important class of synthetic compounds have a variety of applications (Haraguchi S K 2011). These compounds have revealed wide spectrum of activities which includes anticancer (Hu W 2006) , anti HIV (Yogeeswari P 2011), antitubercular (Pitucha M 2011), antiviral (Shipman S H 1981),antitumor (Silva M V 2010), anti-protozoal (Dimmock J R 1991), anticonvulsant (Jatav V 2008), antidepressant (Klayman D L 1979), antimalarial (Yamaguchi H Y 2009), antifungal (Siddiqui N 2003), antibacterial (Chipeleme A 2007) as well as parasiticidal activity against plasmodium falciparum (Wilson H R 1974), Trypanosomacruzi (Greenbaum D C 2007) and Trypanosomabruceirhodesiense (Greenbaum D C 2005) and toxiplasmagondii (Reis C R 2011) Some industrially important activities such as anticorrosion and antifouling effects (Zamani K 2004) and plant growth promoting (Basak S 2010) are also observed for these compounds. These S and N donor ligands and their coordination complexes have gained special attention due to their activity against protozoa, influenza, smallpox virus, fungi and cancer (Khalil M S 2010, Holla B S et al 2003). These compounds arouse interest as versatile intermediates for preparing various heterocyclic derivatives such as 2,4thiazoles (Oruc E E 2004) 1,3, 4 thiadiazoles (Randhavane P V 2010) and 1,2, 4 triazoles (Shi Z G 2011). The copper complexes of thiosemicarbazide and thiosemicarbazones have also been reported (De Silva 2010, Singh N.K 2001) to show antifungal activity towards various fungi.

Collection of Materials

The dried Cannabis sativa (Hemp) seeds were obtained from local market. They are dried in room, clean and stored in a sealed vessel wrapped with polyethylene bag at 40C.

Extraction of oil

After cleaning and removal of the sand and foreign material , the dried Cannabis sativa (Hemp) 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 400C 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.

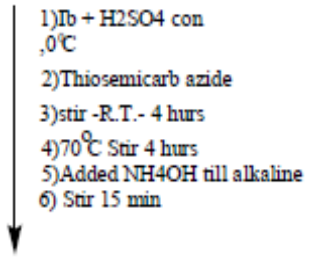
Preparation of Mixed Fatty Acids from oil

Mixed fatty acids from Cannabis sativa (Hemp) 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 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-3-6-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 H2SO4, 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 NH4OH 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 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 290 nm shows absorption 3.6547

Infrared Spectra of EFATSC
The infrared spectra of essential 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 fatty acid thiosemicarbazide (EFATSC) was taken on PW 3710 diffractometer using CuK2 radiation (Y= 1.54060) .The X-RD diffraction of EFATSC recorded at angle 2θ from 10.974 to 39.630. 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 fatty acid thiosemicarbazide (EFATSC) of Cannabis sativa (Hemp) 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 370C 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, Fig 4.

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Table 1.. Infrared Spectra of Essential Fatty acid Thiosemicarbazide (EFATSC) of *Cannabis sativa* (Hemp) seed oil

Sr.No.	Frequency Wave-number	Expected Elements
1)	640	C=S, Stretch
2)	725	Sulfonic acid
3)	750	
4)	950	Ring C-H
5)	1150	R-O-R
6)	1200 1250 1290	$-CH_2$ vibration
7)	1300	O=C=O , stretch
8)	1390	Alkane, tert butyl
9)	1450	Alkanes asy, CH_3
10)	1515	Sec.acyclic amides, amide H bond
11)	1575	N-H plus C=N stretch
12)	1650	N-H det. Plus C-N, stretch
13)	1700	Secondary amide
14)	2500	Charged amines (C=NH)
15)	2550	Organosulphur compounds
16)	3000	N-H Stretch

Table 2. X-RD Spectra of Essential Fatty acid Thiosemicarbazide (EFATSC) of *Cannabis sativa* (Hemp) seed oil

Sr.No.	2θ	hkl	Sin^2 Observed	Sin^2 Calculated	d (A^0) Observed	d (A^0) Calculated
1	10.974	110	0.013874	0.01052	5.4331	5.2351
2	13.146	110	0.01584	0.01384	5.2834	5.19872
3	14.035	110	0.01873	0.01702	5.9731	5.0865
4	14.689	110	0.019987	0.01858	5.1043	4.9987
5	16.273	111	0.02658	0.01434	4.9879	4.5337
6	16.789	111	0.02789	0.01889	4.99897	4.3275
7	17.995	111	0.02995	0.01993	4.89342	3.9938
8	19.015	111	0.03145	0.02147	4.6732	3.7527
9	20.734	200	0.03387	0.02583	4.5041	3.5876
10	21.417	200	0.03958	0.02904	4.5863	3.1632
11	23.656	200	0.04256	0.03285	4.1205	3.0057
12	25.314	210	0.04863	0.03735	3.9987	2.6751
13	26.503	210	0.04995	0.03981	3.8204	2.3889
14	27.879	211	0.05387	0.04355	3.7635	2.0653
15	28.761	211	0.06353	0.04538	3.5049	2.4375
16	29.654	211	0.06798	0.05368	3.0132	2.05561
17	30.335	300	0.06897	0.05993	2.9745	1.9938
18	31.487	300	0.07534	0.06304	2.8638	1.7512
19	31.895	300	0.07876	0.06238	2.8047	1.6329
20	32.603	300	0.08374	0.07112	2.7798	1.4030
21	34.008	310	0.08998	0.07994	2.6325	1.32855
22	35.869	310	0.09103	0.08050	2.4020	1.2067
23	36.157	311	0.09435	0.08135	2.3214	1.1351
24	37.008	311	0.09783	0.08480	2.2004	1.1217
25	38.541	311	0.9931	0.08699	1.9873	1.0429
26	39.630	311	0.9989	0.08935	1.8759	1.0011

a= 7.5698 b= 9.8849 c=8.6547

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Table 3. Antibacterial Activity of Essential Fatty acid Thiosemicarbazide (EFATSC) of *Cannabis sativa* (Hemp) seed oil

Sr.No.	Bacteria	Reference Substance	Inhibition Zone (EFASC) 40 µ / well
1	E.coli	40 + 2.0	12 + 00
2	S.aureus	43 + 1.0	20 + 0.5
3	A.niger	19 + 2.0	14 + 0.5

Figure-1. Absorption Spectra of Essential Fatty acid Thiosemicarbazide (EFATSC) of *Cannabis sativa* (Hemp) seed oil

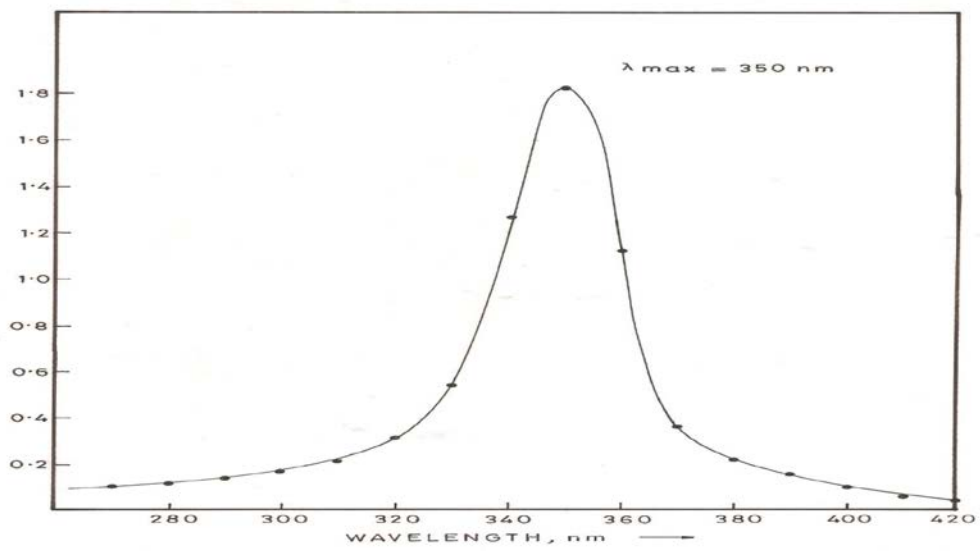


Figure-2 . Infrared Spectra of Essential Fatty acid Thiosemicarbazide (EFATSC) of *Cannabis sativa* (Hemp) seed oil

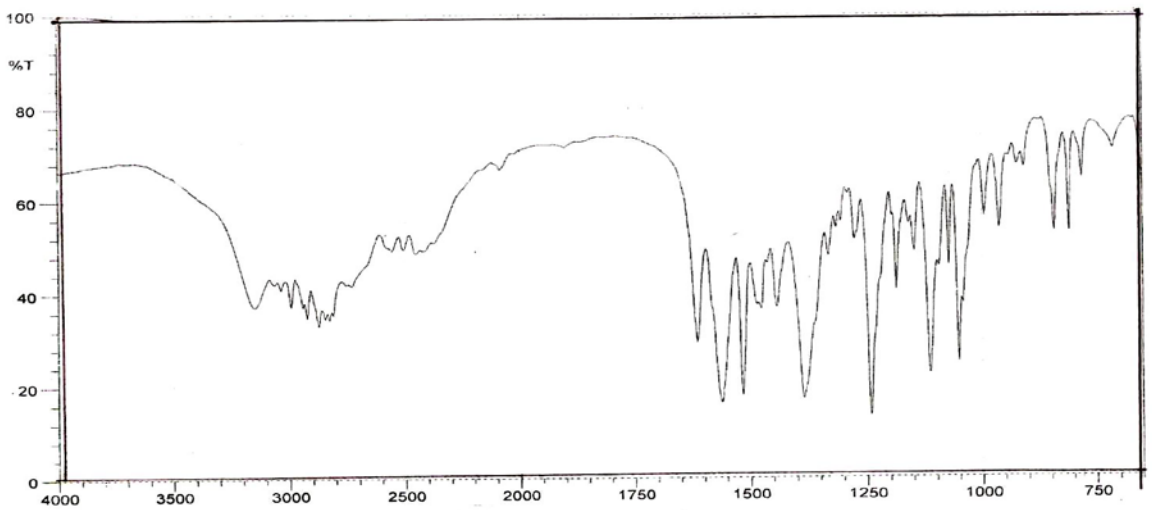


Figure-3 . X-RD Spectra of Essential Fatty acid Semicarbazide (EFATSC) of *Cannabis sativa* (Hemp) seed oil

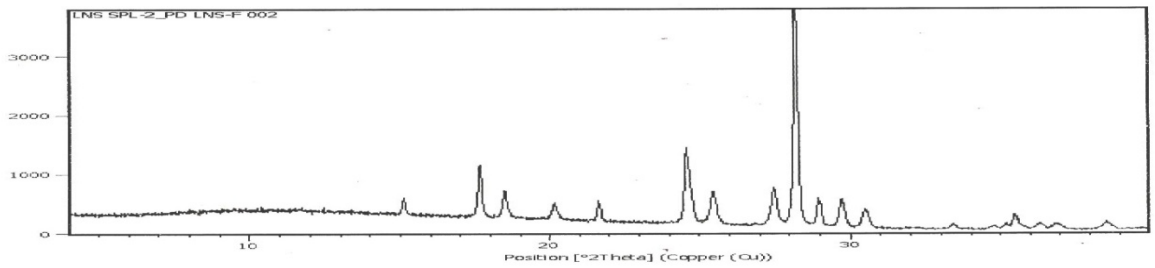
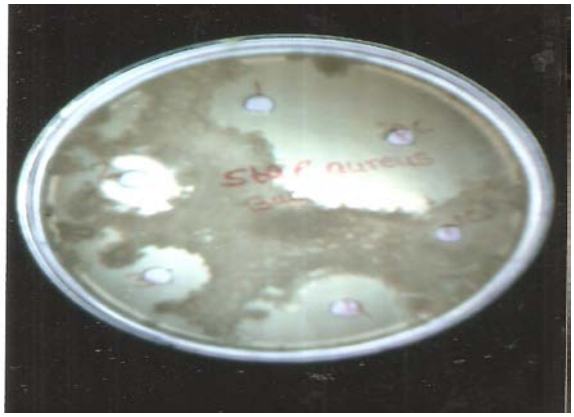


Figure-4. Antibacterial Activity of Essential Fatty acid Semicarbazide (EFATSC) *Cannabis sativa* (Hemp) seed oil



Conclusion

Cannabis sativa (Hemp) seed oil is bold yellow colour with pleasant nutty odour, paint like odour. Hemp seed contain oil 32.21 %, moisture 03.07 % , protein 23.90 % , specific gravity (25 ^{0}C) 0.8927, refractive Index at 40 ^{0}C is 1.4570, acid value 2.15 mg KOH / g of oil , saponification number 190.2 mg KOH / g of oil .It 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 *Cannabis sativa* (Hemp) seed oil is used a medicinal important. Absorption spectra of essential fatty acid thiosemicarbazide (EFASC) of *Cannabis sativa* (Hemp) seed oil shows maximum absorption 3.6547 at 290 nm.Infrared spectra of EFASC shows that at 640 cm^{-1} C=S, Stretch, at 950 cm^{-1} ring C-H, at 1150 cm^{-1} R-O-R, at 1250-1290 cm^{-1} CH_2 vibration, at 1390-1450 cm^{-1} Alkanes CH_3, at 1575 cm^{-1} N=H plus C=N stretch, at 1650 cm^{-1} N-H & C-h stretch, at 1700 cm^{-1} secondary amide, at 2500 cm^{-1} charged amines C=NH, at 2550 cm^{-1} organosulphur compounds, at 3000 cm^{-1} N-h stretch. X-RD spectra of essential fatty acid thiosemicarbazide (EFATSC) of *Cannabis sativa* (Hemp) seed oil indicate a=7.5698 , b=9.8849 & c= 8.6547 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 was found to be more active in inhibition zone than E coli & A.niger. The result calculated that EFATSC of *Cannabis sativa* (Hemp) seed oil posses good antibacterial activity.

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