IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 2 Ver. I (Mar. – Apr. 2018), PP 21-31
www.iosrjournals.org

Isolation And Charaecterisation of Compounds Produced By Bacillus Starins From Soil And Determination of Antiinflammatory Activity

E. Sam Jeeva Kumar[1*], Dr.M Ravi Kumar[2], T. Vimal Kannan[3], P.Venkata Ramana[4] , S. Parveen[4] S.Md.Yaseen Shahzadi[5], S. Susmitha[5]

1- Research Scholar, JNTUH, Hyderabad.
2- Principal, Githanjali collage of pharmacy, Hyderabad.
3,4,& 5- Department of pharmacy practice PRRM Collage of pharmacy Kadapa.
Address for correspondence:
E. Sam Jeeva kumar
Associate professor
P.Rami reddy memorial college of pharmacy
Utukur,Kadapa. pin: 516003
Corresponding Author: E. Sam Jeeva Kumar

Abstract: *The soil is considered as the region of earth crest was geology and biology meat. There are two category of soil namely mineral soil which contains solid matter in the region and organic solid which contain rich amount of organic matter. Bacillus are rod shaped bacteria which consist of two genera namely aerobic bacilli an aerobic bacilli. Bacillus organism produce a wide range products like antibiotic, enzymes and insecticides. The soil is collected from the mountain region of south India primary and secondary screening were done. The resulted organism were subjected to fermentation by LG medium. The form the product was subjected to analytic characterization. Also the test for anti inflammatory activity was carried out. The morphological and biochemical characterization were done for the organism. A rod shape bacteria was seen on viewing through a light microscope. The submerged fermentation and solid state fermentation produce aezulene, pergenenediol and ethyl iso allocholate. The compound showed excellent anti inflammatory activity. Economically this compound showed useful results of anti inflammatory activity.*

Keywords: *Soil, bacillus strains, anti inflammatory activity.*

Date of Submission: 27-02-2018 Date of acceptance: 12-03-2018

I. Introduction

Soil as been defined as the region as earth's crest were geology and biology meet. From a functional view point the soil may be considered as the land surface of the earth which provide the substration for plant and animal life. The characteristics of the soil environment varry with the locale and climate. Soil differ in depth, physical properties, chemical composition and organisams. Generally there are two types of soil namely Mineral soil, in which the solid matter present is inorganic &Organic solids those which have very little inorganic matter. Fertile soil consist of root system of higher plants & many animal form like rodents, insets,& worms. It also contains a large numbers of microorganisam. The differences in the compositions of soil along with their differences physical characteristics and agricultural practices by which there are cultivated results in a large differences in the microbial populations both in total numbers and kinds. The condition present in the soil influence the growth of microorganism in the laboratory. The condition which the microbes need for the growth with reference to the soil are

1. Amount & type of nutrients.
2. Available moisture.
3. Degree of Aeration.
4. Temperature.
5. pH.
6. Practice and occurrences which contribute large number of micro organisms in the soilThe existence of roots & extensiveness of root systems in the soil also influnce the number and kinds of microorganism present in the soil.

Because of the variation in the climatic conditions also influnce certain physiological types. Interaction among microbial species or also has an important effect on microbial population. These is an extensively complex situation . Predatory protozoa and antibiotic producing actinomycetes may eliminate certain group of microorganism. Few environments on the earth have a great verity of microorganisms as fertile soil.

Bacteria, Fungi,Algae,Protozoa and Viruses makeup this microscope population which may reach a total billions of microorganism per gram. The great diversity of microorganism makes it difficult to determine accurately the total numbers of microorganism present. Culture method will give information on the physiological and nutritional types compatible with the cultural environment direct microscope method give information about all microorganisms except viruses, but this technique as limitations in determine the living microorganism from the death. Very often the microbiological analysis of soil is concerned with theisolationand identificationof specific physiological types of microorganism. For this purposes enrichment culture technique are appropriate. Sprogenus, Rod shaped Bacteria are classified to Genera the Aerobic Bacilli and the aerobic clostearidia. The genus bacillus consist aerobic bacilli forming heat resistance spores.There are gram Positive, but tend to be decolourised easily so as to appear Gram variable are even frankly gram negative there generally motile with pertrichous flagella, the anthrax Bacilliusbeening a notable expection. Members of this group often exhibit in there properties.The genius include psychrophilic, mesophilc thermophilic speacies, the maximum tempeture for vegetative ranging from 25º-75ºC and minimum from 5º-45ºC.The salt torlance verries from less than 2%-25% sodium chloride. Bacillus spores are ubiquitous been fount in the soil, Dust, water and air which constitute the common contamints in bacteriological culture media. Bacillius Anthraces is a causative agent for anthraces, which is the mager pathogenic bacteria. Bacillus cerus make causes gastroenteritis. Some Bacillius spaces are responsible for opportunistic infections. Bacillius cerus distribute widely in nature and this can be isolated from soil, vegetables and wide verity of foods containing milk,cereals, meat and poultry. Bacillius cerus is generally motile, but nonmotile strains may occur. It resemblesbacillus anthraces except that it is not capsulated and not susceptible to gamma phage and does not react with anthrax florescent antibodie. Fermentation involes microbial metabolism to transfer simple raw materials to valuble products. The ability of the organism to growth in a varity of substrates and there by producing wide range of products only reflects their biochemical diversity, but also resulted in their commercial exploitation by the fermentation industry. Fermentation is an energy generating process in which organic compounds act both electron donors and electron acceptors. Micro organisms conducts a wide verity of metabolic procesto option energy and new cell material required there growth and multiplication. A few organisms i.e, Photosynthetic are able to utilize light energy to covert carbon dioxide(co_2) from air are hydrogen from water into cellular organic material. The common industrial organism require organic substrates for growth.

During metabolism to appositive at the same time indivisible process occur , constructive and energy metabolism. Constructive metabolism processed with the absorption of free energy. For this type of metabolism, a compare to a small amount of food material gives by the cell is expended. Energy metabolism serve for the conversation of energy to a form on which it is utilizes by the cell. For this purposes a large amount of nutrient is used. This two process can not be separated and the interconnected. Products of incomplete oxidation of the substrate are valuable to the organism, not only change it's energy sources, but are compounded parts which are used for the cell. Metabolism is carried with the help of enzymes. Generally fermentation can be classified into two which are submerged fermentation and solid state fermentation. Submerged fermentation is carried out by means of bioreactors which are made of stain eases stain and provided with mechanical internal agitators for mixing content in the bioreactors.NCultivation progressively involves the suspension growth of microorganism in liquid environment. Solid state fermentation are governed both microbial growth and products formation.

Predominately takes place at the surface of solid substrate. The genus bacillus includes the wide verity ofindustry important microbial spaces which are commonly use in fermatation industry. Bacillus spaces can be cultivated in extrame conduction of temperature and $P_H$to give product that are stable in the wide verity of harsh environment. The large divergence in physiology types presents in Bacillus spaces have attributed to the great diversity in the genus and most members are non pathogenic,relatively is easy to manipulate by genetic, good secretors of protons and simple to cultivate which makes bacillus one of the hosts for fermentation. Bacillus spaces also sporulate and its impact is necessary to be considered for product formation. Bacillus spaces are used for the production of antibiotic, enzymes and insecticides. Here we report the production of pregnendiol and azulune compounds, phenentharine using bacillus spaces.

II. Material And Methods

The soil is collect from the mountain regions of south India.

Primary screening:

1% solid solution is inoculated into nutrient ager media.

The composition of nutrient agar media is as follows

Peptic degust of animal tissue ------5gms

Beef extract ----------1.5gms
Yeast extract ----------1.5gms
Sodium chloride----------5 gms
Agar----------15gms
Final Ph (25ºC)----------7.4 +/- 0.2.

Secondary screening:
Selective screening of bacillus spaces is carried out as follows

INGREDIENT	**Gms/ Lit**.
1. Dipotasium hydrogen phosphates	1.00
2. Magnesium sulphate	0.200
3. Sodium chloride	0.200
4. Ferrous sulphate	trace
5. Soil extract	5.00
6. Agar	15.00
7. Final PH	8.3(25ºC).

IDENTIFICATION OF BACTERIA:
Simple staining:
Smear is stain with methylene blue.
Grams staining :
The bacterial smear is fixed on glass slide. Primery staining was done with crystal violate a dilute solution iodine was applied. Discoloration was done using acetone. Counter stained with carbol fushion
Biochemical reaction:
The biochemical text for indole production, methyl red, Vogus-proskaur text, cittrate production, urease text, Actalast production, oxidase reduction, Hydrogen sulphate reaction was carried out.

SOLID AND SUBMERGED FERMENTATION:
The fermentation was done using LG a medium in a rotary shaker at 120 rpm.
Varied temperature and PH were used to optimized the production of the biomass.
The temperature were varied from 27ºC to 47ºC and PH were varied, 5,6,9.
All this parameters were done by altering one parameter at the time by keeping others at a contain level.

The composition of LG medium is as follows

INGREDIENDS	**Grms/Lit**
1. Potassium di hydrogen phosphate	10gms
2. Yeast extracts 0.5 gms	
3. Di potassium hydrogen phosphate	0.06 gms
4. Magnesium sulphate	0.02 gms
5. Calcium chloride	0.02 gms
6. Ferric chloride	0.002 gms
7. Sodium molybdate	0.002 gms.
8. Ph was adjusted to 6.8 using 1M sodium hydroxide.	

POTIMISATION OF AERATION AND AGITATION:
With the help of 3L fermenter oxygen supply and the effect over the production of biomass as been analysis The working volume of the reactor was found to be 2.7L. Air flow was varied from 0-6 volume of air / volume of liquid / min. Triplicates of 1, 2,3,4,5 and 6 vvmtaken. This all are oxygen contact was analysed by oxygen sceneries of the fermenter. Selective LG medium was employed for batch fermentation process.

EFFECT OF AGITATION:
By varring the speed of the staried from 100-500 RPM the corresponding biomass productions was measured. Triplicates of 5 readings were taken at 100,200,300,400 and 500 RPM. The optimized values of other studies was varied to optimized the yield of biomass production.

ANALYTICIN CHARECTERISATION:
1. DETERMINATION OF OPTICAL DENSITY:
The optical density was calculated using SHIMADZU UV SPECTRO PHOTOMETER.

2. IR SPECTROSCOPY:

1 mg of biomass was mixture with potassium bromide in an agate morter and spessed at high pressure of 2500 psi to form a pallet was used for analysis.

INSTRUMENT NAME: SHIMADZU FTIR SPECTRO PHOTOMETER.

GC MS CONDITION:

INSTRUMENT NAME: JEOL GC METE II.
Flow innet temperature: 220ºC.
Colum: HP5 Ms.
Carrier gas: High pure Helium.
Flow rate: 1 ml/min.
Oven temperature: 50-250ºC at 10º/min.
Ion chamber temperature: 250ºC.
GC Interface temperature: 250ºC.
MASS ANALYSIER:
Quadruple double focusing mass analyzer.
Detector for gas promotography photo multiplier tube.
Scan: 50-60 amu ‘ 70 ev
Ionization : electron impact ionization.
NMR SPECTROSCOPY:
PMR and C_{13} Spectra was recorded at 103 MH_Z and 297 MH_Z and bruker AM 300 spectrophotometer.
Spectro was recorded at 5mol/lit solution in Me OD. At ambient temperature. Chemical sifts were expressed in parts ppm relative to the external tetramethyl xilane.

DETERMINATION OF ANTI INFLAMATORY ACTIVITY:

The biomass is insoluble in water and it’s made soluble in corboxcial methyl cellures at a concentration of 60 mg in 15ml of water and triturated in motal and pestle. 4 sets, each consisting of 3 animals of wister albino female rats were selected. Carragenin (1ml-3mg standed induce paw edema method was done). Reading was taken for every 30 min, 60 min, and 90min were taken. To readings were taken from drug extracts and 1 reading was taken from the control.

This study was approved by the animal ethics commity of P.RAMI REDDY MEMORIAL COLLAGE OF PHARMACY KADAPA with the approval No: 1423/PO/a/11/CPCSEA 2016.

III. Results

By morphlogicin identification a rod shaped bacteria was found. The biochemical identification showed the following characteristics.

Indole: -ve , Methyl red: -ve, Nitrate –+ve , Vogus proskar : -ve, Citrate: -ve, Catalase: +ve, Triple sugur iron : -ve, H_2S : -ve, Gas : -ve, Gram staining :

Submerged fermentation (Table 1):

PH

PH	24 Hours	48 Hours	72 Hours
5	0.055 U/ml/min	0.072 U/ml/min	0.036 U/ml/min
7	0.060 U/ml/min	0.070 U/ml/min	0.031 U/ml/min
9	0.051 U/ml/min	0.060 U/ml/min	0.028 U/ml/min

Temperature

Temperature	24 Hours	48 Hours	72 Hours
27°C	0.059 U/ml/min	0.054 U/ml/min	0.061 U/ml/min
37°C	0.046 U/ml/min	0.0041 U/ml/min	0.046 U/ml/min
47°C	0.035 U/ml/min	0.031 U/ml/min	0.028 U/ml/min

Effect of sodium chloride

Sodium chloride%	Bacilli
0.5%	0.050 U/ml/min
1%	0.076 U/ml/min
1.5%	0.055 U/ml/min
2%	0.043 U/ml/min

UV Exposure

Hours	Minute	OD value
24 Hours	10 Minute	1.628
	20 Minute	1.44
48 Hours	10 Minute	1.208
	20 Minute	1.089
72Hours	10 Minute	1.510
	20 Minute	1.128

Solid state fermentation (Table 2):

PH

PH	24 Hours	48 Hours	72 Hours
3	1.388 gms/hr	1.078 gms/hr	0.928 gms/hr
5	1.476 gms/hr	1.128 gms/hr	0.854 gms/hr
7	0.948 gms/hr	0.728 gms/hr	0.703 gms/hr

Temperature

Temperature	24 Hours	48 Hours	72 Hours
27°C	0.168 gms/hr	0.321 gms/hr	0.467 gms/hr
37°C	0.519 gms/hr	0.262 gms/hr	1.017 gms/hr
47°C	0.398 gms/hr	0.454 gms/hr	0.835 gms/hr

Salinity

Percentage	
0.50%	0.853
1%	0.957
1.50%	0.789
2%	0.986

Nitrogen source

	24 Hours	48 Hours	72 Hours
Ammonium chloride	**1.201** gms/hr	**0.94** gms/hr	**0.815** gms/hr
Ammonium dihydrogen phosphate	**1.038** gms/hr	**0.873** gms/hr	**0.713** gms/hr

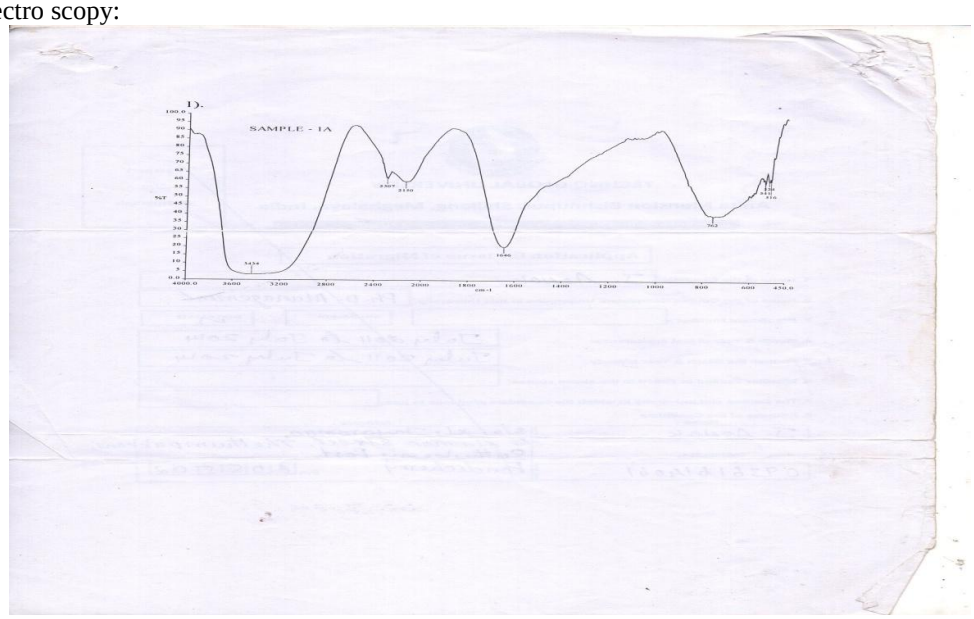
Carbon Source

	24 Hours	48 Hours	72 Hours
Lactose	1.02 gms/hr	0.978 gms/hr	0.78 gms/hr
Maltose	1.011 gms/hr	0.861 gms/hr	0.753 gms/hr.

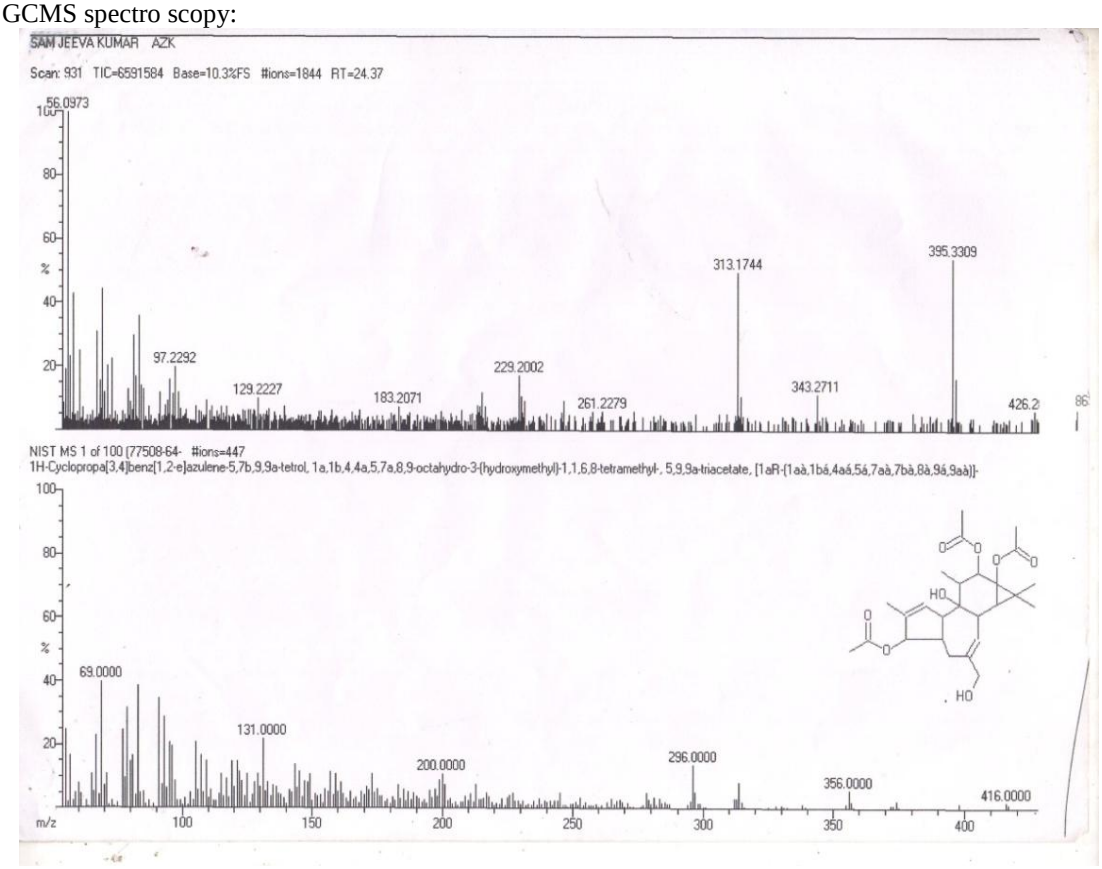
Uv exposure (Table 3):

Analytical studies:

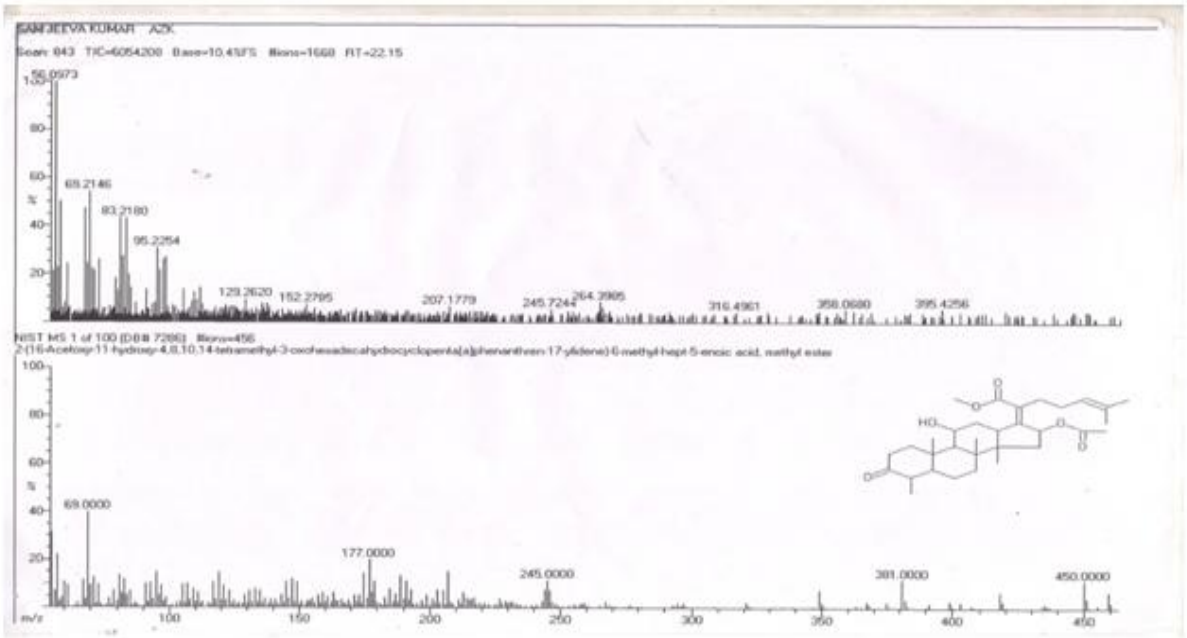
1. IR spectro scopy:



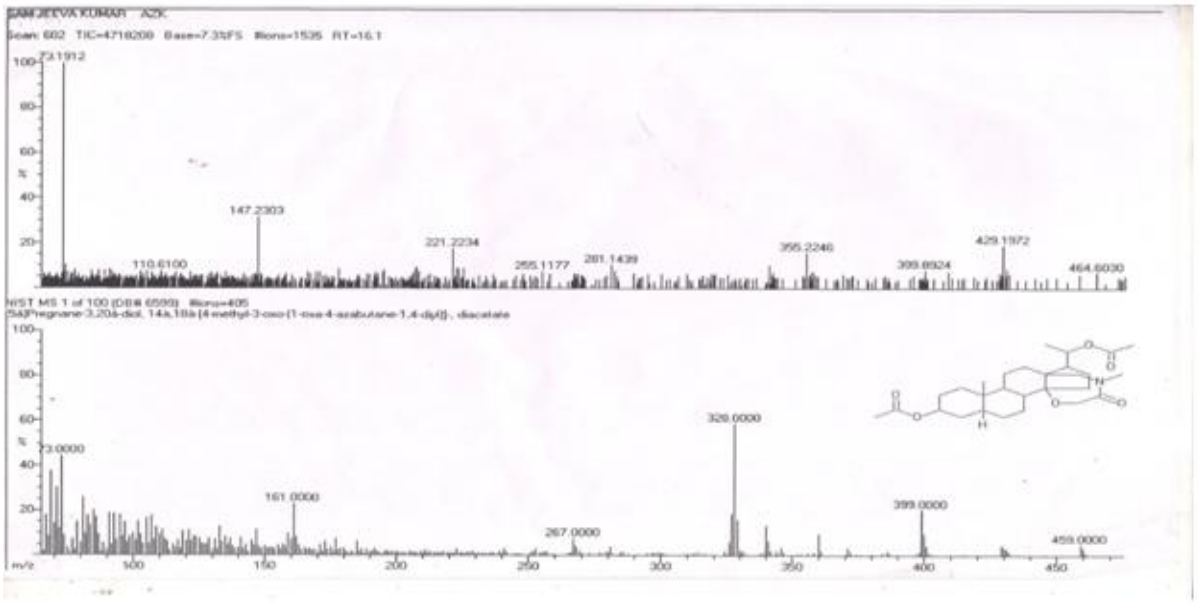
2. GCMS spectro scopy:



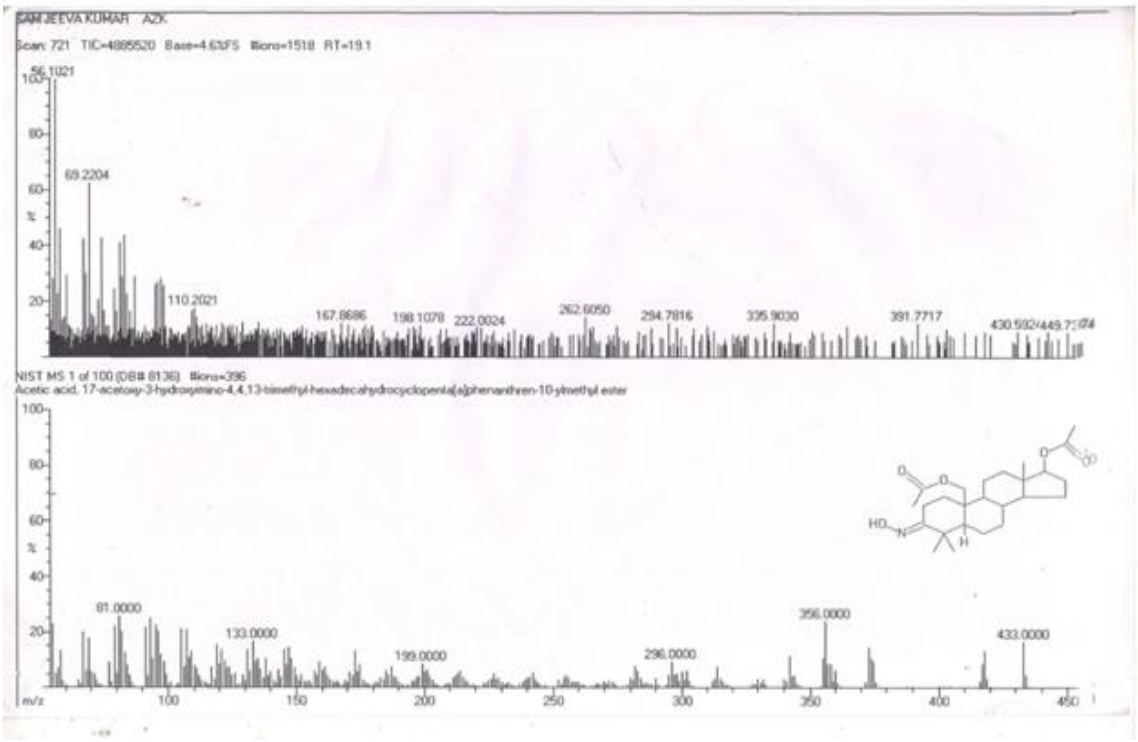
Spectram 1



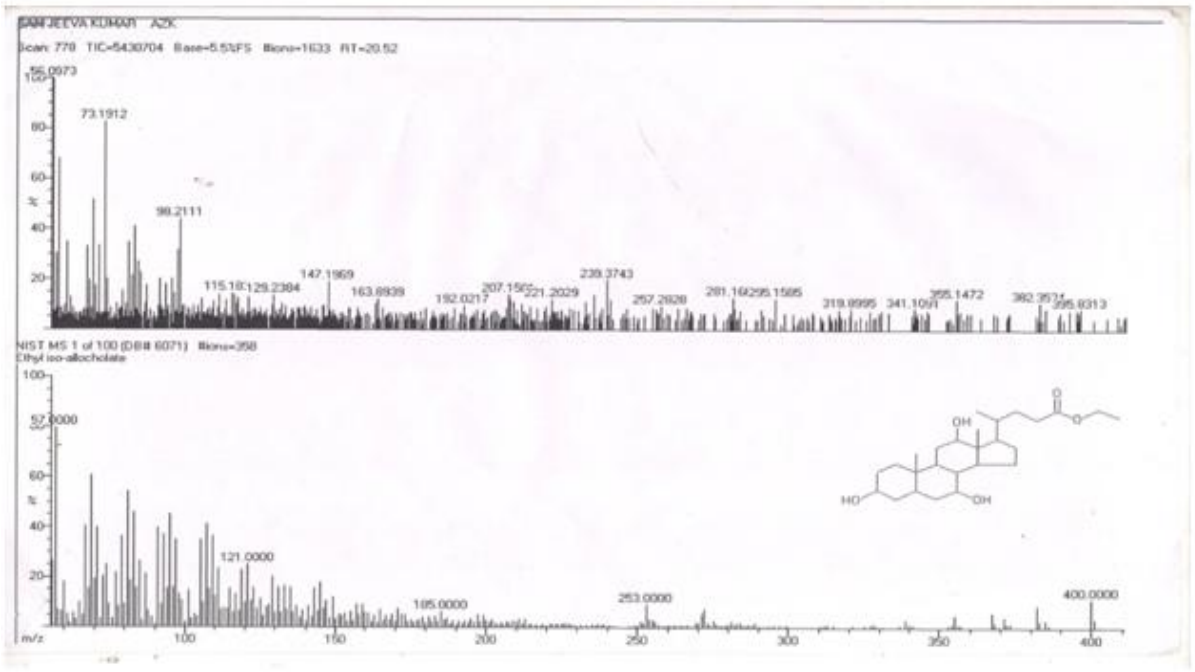
Spectram 2
B



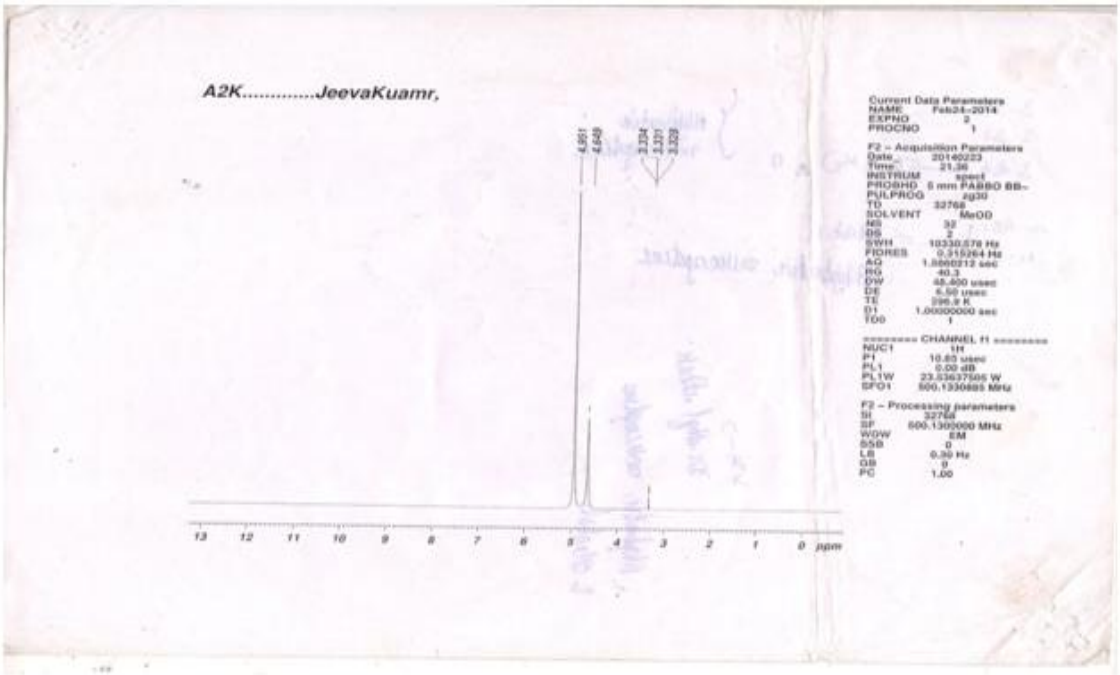
Spectram 3



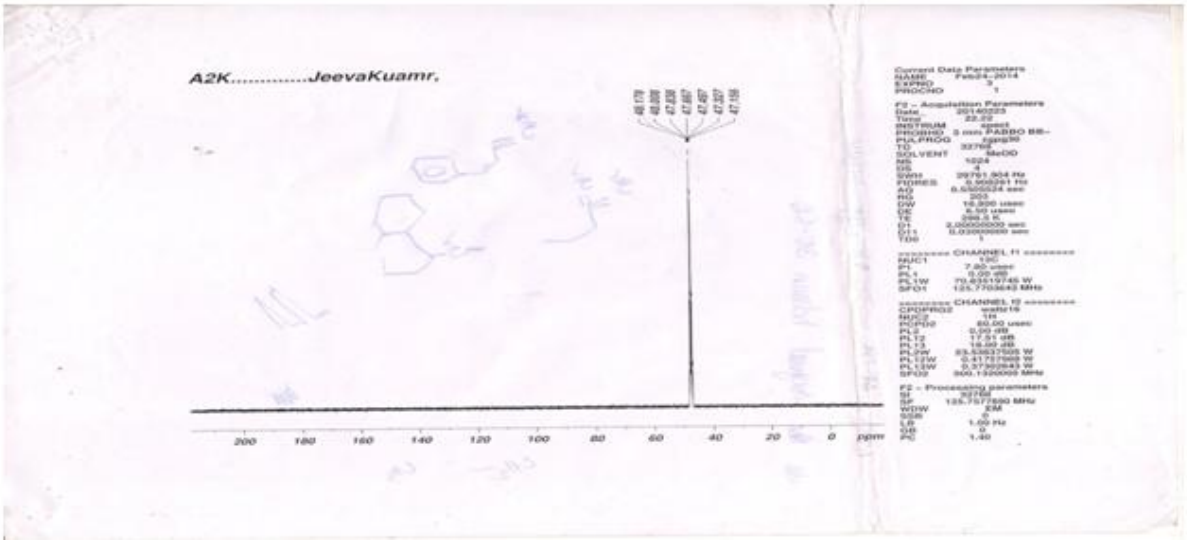
Spectrum 4



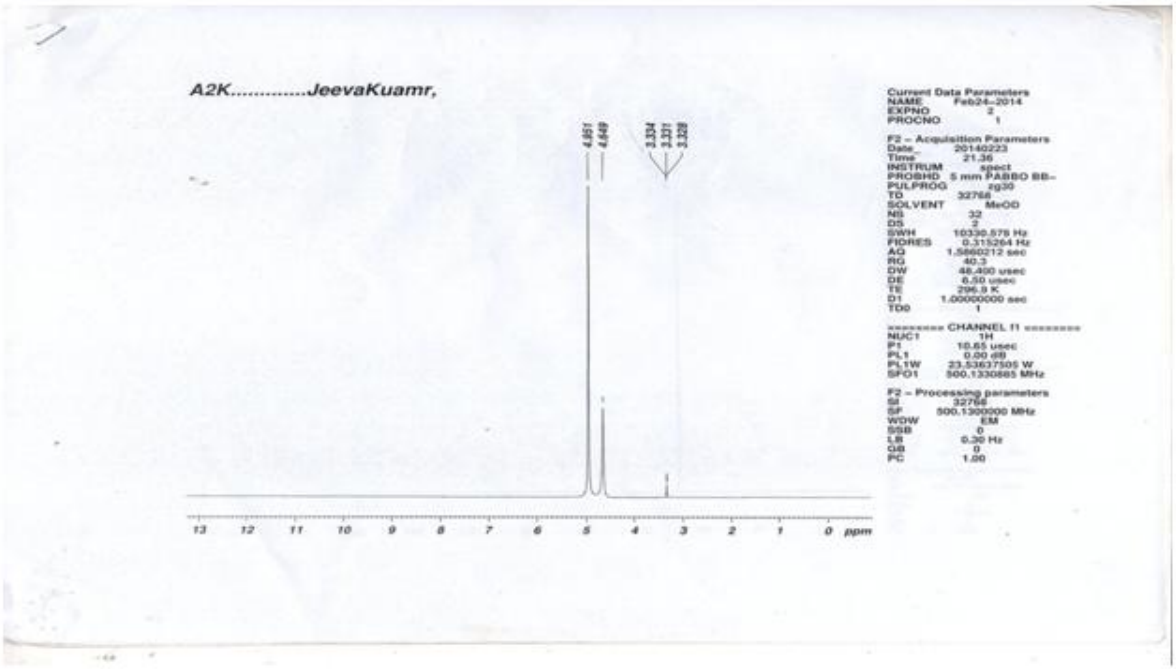
Spectrum 5
NMR spectroscopy:



Spectrum 6



Spectrum 7(C_{13}NMR SPECTRO SCOPY)



SPECTRUM 8.
Determination of anti-inflamatatory activity:
SET-I

ANIMAL	Omins	30mins	60mins	90mins
1	2.5cm	2.7cm	2.2cm	2.1cm
2	2.4cm	2.5cm	2.3cm	2cm
3 (control)	2.4cm	3.1cm	2.9cm	2.9cm

SET-II

ANIMAL	0mins	30mins	60mins	90mins
1	2.6cm	2.7cm	2.3cm	2.1cm
2	2.5cm	2.5cm	2.2cm	2.1cm
3 (control)	2.5cm	3cm	2.8cm	2.8cm

SET-III

ANIMAL	0mins	30mins	60mins	90mins
1	2.7cm	2.8cm	2.2cm	2.1cm
2	2.6cm	2.6cm	2.2cm	2.1cm
3 (control)	2.5cm	3cm	2.8cm	2.8cm

SET-IV

ANIMAL	0mins	30mins	60mins	90mins
1	2.4cm	2.6cm	2.2cm	2.1cm
2	2.4cm	2.5cm	2.1cm	2.0cm
3 (control)	2.4cm	2.6cm	2.5cm	2.5cm

IV. Discussion

The bacillus organisms are rod shaped bacteria and it's identified by simple staining. By gram staining they are gram positive but they tend to appeared gram negative. Biochemical identification illustrates that bacillus organism fermented glucose, maltose, and sucrose and the fermented producing acid but, no gas. Nitrates are reduce to nitrites. Catalase is formed. Bacillus is species is formed are known to be inhabitant from soil and can with stain both high and low temperature condition. Light microscopic studies permits studies on the instation of colony formation.While carrying out submerged fermentation the yield was found to be maxium at P_H-48 Hrs (0.070 U/ml/min) and the yield decreases 72 Hrs(0.031 U/ml/min). Also the maxium yield was found at 27^{o}C at 72 Hrs(0.061U/ml/min). At elevated temperatures it was found that the yield was decreasing with the effect of sodium chloride the maximum yield was founded 1% of sodium chloride with the increasing the concentration of sodium chloride the yield was found to be decreased also with UV a maximum value of appetencies density was founded 24 Hrs 10min and then the value started decreasing. Since bacillus organism utilize sucrose as a carbon source the maxium yield was got as 24 Hrs at PH 5 utilizing sucrose as a carbon source. When using lactose and maltose the yield was found to be decrease.When using yeast extract as a nitrogen source the yield was found to be decrease. When the nitrogen source was substuted with ammonium chloride and ammonium di hydrogen phosphate the yield was found to be decrease. With regard to temperature the maximum yield was obtained at 37 ^{o}C the yeid was found to be maximum with the increase temperature the yield was found to be decreases. IR spectroscope reveals at 3434cm^{-1} a OH group was present. At 2307,2150 cm^{-1} an amino group was present. At 1646 cm^{-1} C= C stretching, moderate to week absorption. At 762 cm^{-1} C-H bending vibration are present.

INTERPRETATION OF MASK SPECTRUM:

Spectrum 1:

Show the base peak at m/z value is 69 and the molecular ion peak at 416.

m/z value 131 $C_3H_3N_2O_4$ m/z value 200 $C_8H_{12}N_2O_4$m/z value 69 C_3H_3NO

Spectrum 2:

As a base peak at m/z value 69 and the molecular ion peak at 450.m/z value 69 C_3H_3NO m/z value 177 $C_6H_6NO_4$ m/z value 245 $C_{11}H_{21}N_2O_4$.

Spectrum 3:

As a base peak at m/z value 57 and the molecular ion peak at 450. m/z value 57 C_2H_3NO m/z value 121 $C_2H_5N_2O_4$ m/z value 185 $C_7H_9N_2O_4$.

Spectrum 4:

As a base peak at m/z value 69.2204 $\rightarrow C_5H_9$. Molecular ion peak at 430.532 m/z value :110.2021 C_8H_9, m/z value :187.8636 $C_{14}H_{19}$., m/z value :198.1078 $C_{14}H_{14}O$, m/z value :222.0024 $C_{15}H_{10}O_2$.

Spectrum 5:

At a base peak 73.1912 $\rightarrow C_4H_{11}N$, Molecular ion peak: 454.6030, m/z value : 110.6100C_8H_{14}, m/z value :147.2303 $C_{10}H_{13}N$, m/z value :221.2234 $C_{14}H_{25}N$

Spectrum 6:

At a base peak 73.1912 $\rightarrow C_4H_{11}N$, Molecular ion peak: 464.6030,m/z value : 110.6100 C_8H_{14}, m/z value :147.2303 $C_{19}H_{15}$, m/z value :221.2234 $C_{14}H_{32}$.

In H^1 NMR (proton magnetic resonance spectroscope):

wo singles are observed at ppm 4.951. It may be due to ROH AND AT 3.34 It may be due TO H-C-OR.

With regard to anti-inflammatory activity the compound showed excellent anti-inflammatory activity at 60min and 90 min after application. From this it can be control that compound can be considered for anti inflammatory activity.

V. Conclusion

When seeing economically bacillus organism produces fermentation products with more economical value and with excellent anti inflammatory activity. So, Hence this products can be used medicinally.

The strain isolated was found to have a potential source of production of steroid compounds. The strains also produce constable amount of the compound at the ambient temperature, PH and other environmental factors. The strains also produce maximum yield at 37^{o}C which is worth considering also its ability to with stained alkalinity is also worth considering.

References

[1]. MICHAEL .J.BELCZAR, Jr,E.C.S. CHAN, NOEL R. KRIEG, Microbiology, TATA Mc GRAWHILL publishing company, NEWDELHI 26 reprint 2004 pages 544 – 545.
[2]. R. ANANTHA NARAYANA C.K JAYARAM PANIKAR, text book of Microbiology, UNIVERSITY'S press Hyderabad 8th edition 2009 pages 242 -245.
[3]. Dr. CHANDRAKANT KOKARE, pharmaceutical biotechnology, NIRALI PRAKASHAL PINE, 3rd sep 2013 pages 3.1 – 3.4.
[4]. ROBERT SILVERSTAIN, FRANCIS X WEBSTAIR, spectrometric identification of organic compounds, wiley India (P) ltd, NEW DELHI 6th edition, 3005, pages 45 – 55.
[5]. Y. R SHARMA, elementary organic spectroscopy, S. Chand and company NEW DELHI 4th edition reprint 2009, pages 182 – 253.
[6]. WWW. WICKEPEDIA COM.
[7]. M. V ARBIGAL, B. A. BULTRUS, J.SCHULTZ, D. CRASS, fermentation of bacillus.
[8]. A SOWJANYA, K. RAM PRASAD, ISMAIL, evaluation of anti inflammatory and analangesic activity of methalnolic extract of TERMINALIA Alatabark in experimental animals, international journal of pharmacological screening methods, volume 7 issue 1, 2017, pages 6 – 12.
[9]. FARUK ADANU KUTA at al. screening of bacillus species with potentional of antibiotic production, applied medical inflammatic, volume 24 no 1- 2/ 2009, pages 42 - 46.
[10]. A. G. O' DONNELL et al. Charataritation of bacillus subtilis, bacillus pumulis, bacillus licheniformis, bacillus amylo liquiformis by pyorolisis gas liquid chromoto graphy, Deoxy ribo nuleic acid – Deoxy ribo nuleic acid hybridization, biochemical test and A P I systems, international journal of systemic bacterialogy, April 1980, pages 448- 459, volume 30, No-2.

E. Sam Jeeva Kumar "Isolation And Charaecterisation of Compounds Produced By Bacillus Starins From Soil And Determination of Antiinflammatory Activity." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.2 (2018): 21-31.