Wetland colonizers to anti dandruff dermatitis: study with swamp cabbage

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PREVIEW

PEOPLE-PLANT INTERACTION; Ayurveda and Agriculture

ALLELOPATHY

Ipomoea aquatica Forsk.

- **Origin** : Exotic Aquatic Macrophyte.
- **Habitat** : Semi Aquatic; Aquatic; Tropical Plant.
- **Use** : Green Vegetable (most commonly).

AQUATIC NUISANCE

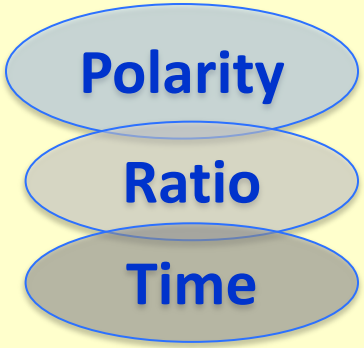
Class A noxious weed, growth rate of approximately ***4 inches per day*** with trailing vines and milky sap

Ipomoea-ETHNO-BOTANIC MEDICINE

INDIA	**: Jaundice, Nervous disability.**
SRILANKA	**: Liver & Eye disorder.**
CAMBODIA	**: Ringworm infection fever & to treat asthma.**
CHINA	**: Costiveness, Putrefaction of the skin & Uterus, Food poisoning.**
INDONESIA	**: Asthma, Boils & Haemorrhoids.**
PHILIPPINES	**: Diabetes.**

The NAPRALERT (NAtural PRoducts ALERT) database

METHODOLOGIES



Collection and processing of plant samples

Extraction of the desired Compound

Isolation and Identification of the Allelopathic Biomolecules

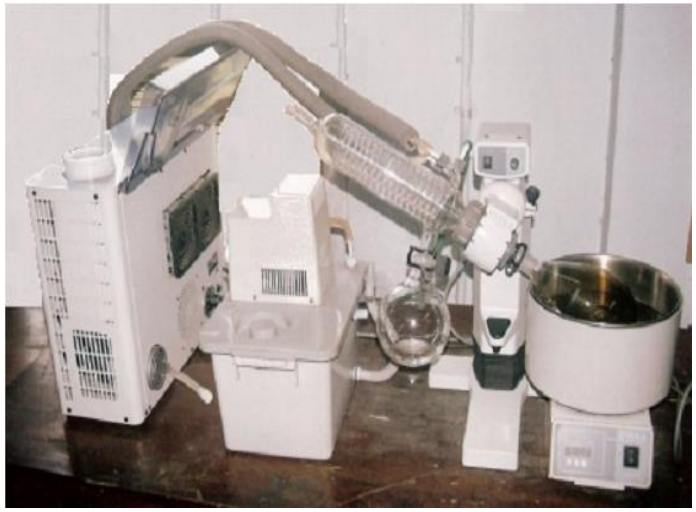
Antimicrobial activity of the pure isolate

Fresh weight: 7.2kg (approx.)
Dry weight: 3.1kg (approx.)

Collection site: Akaipur wetland (West Bengal)
22.9354 latitude; 88.7428 longitude

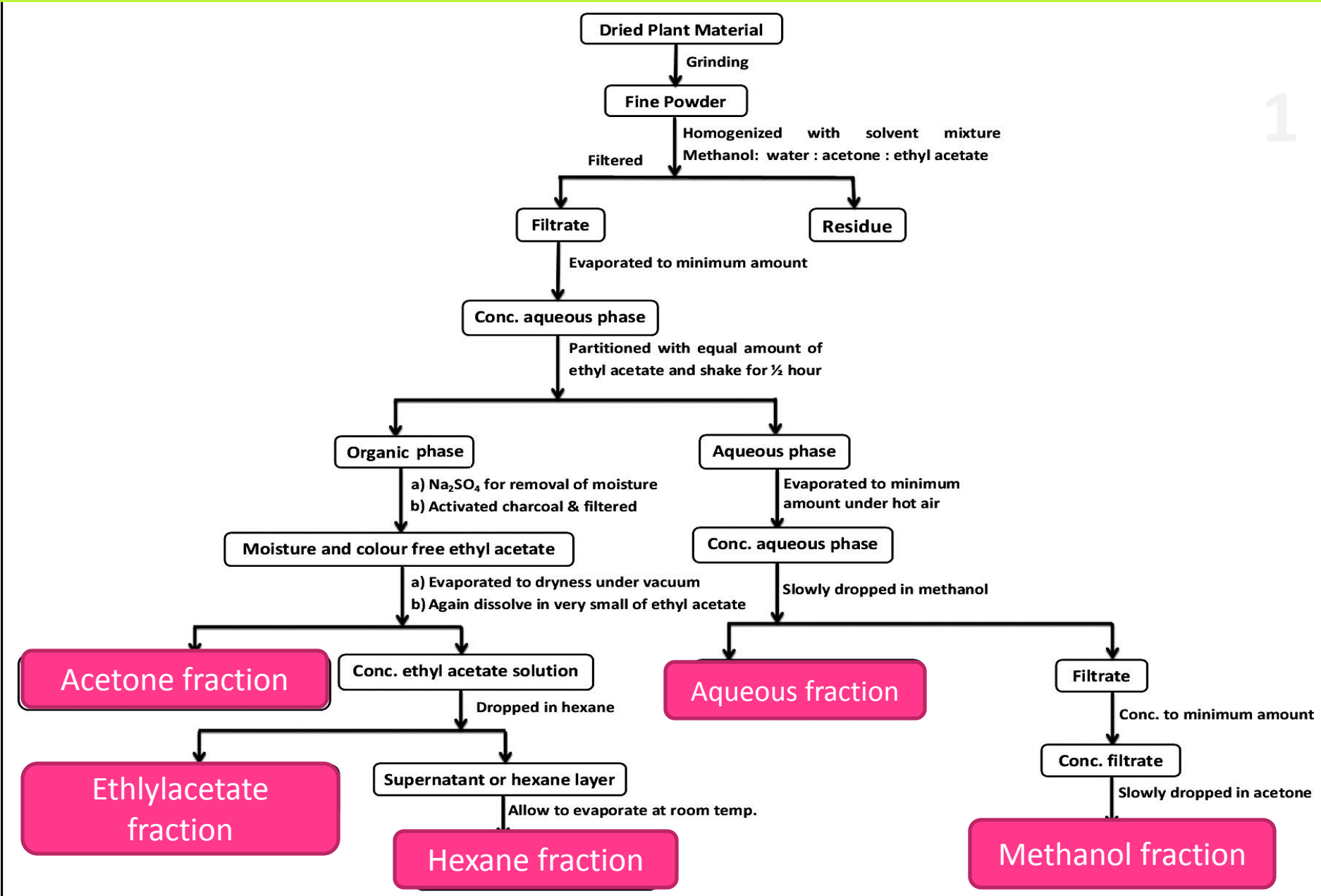
Mechanical Stirrer

Sintered disc funnel with vacuum pump



Rotary Vacuum Evaporator

LIQUID-LIQUID EXTRACTION

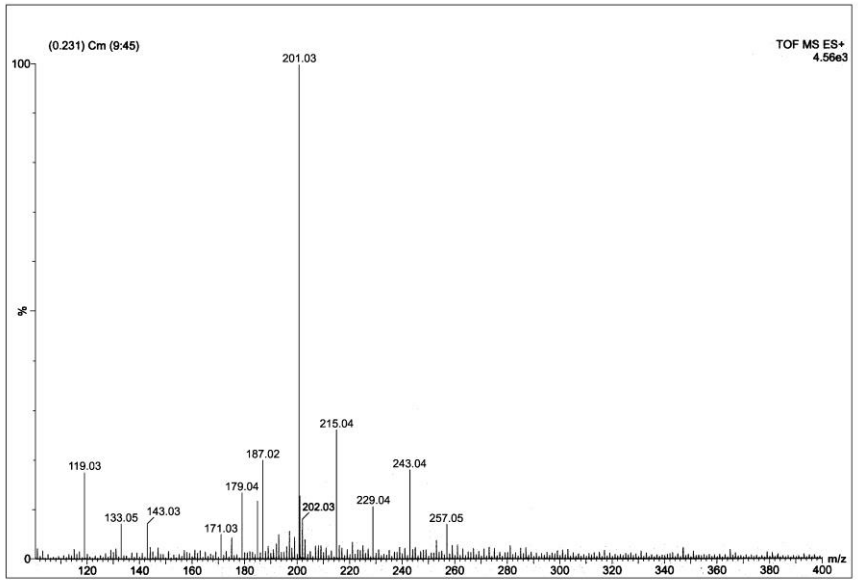


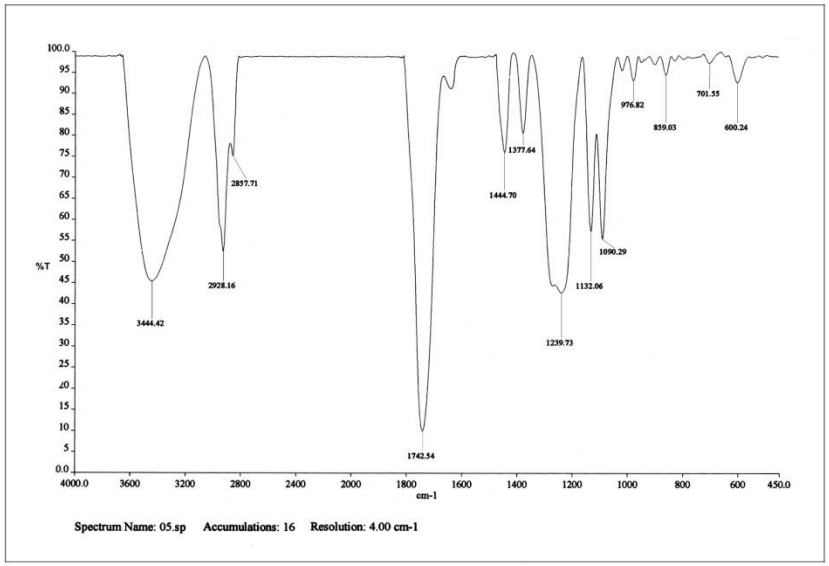
Chromatography

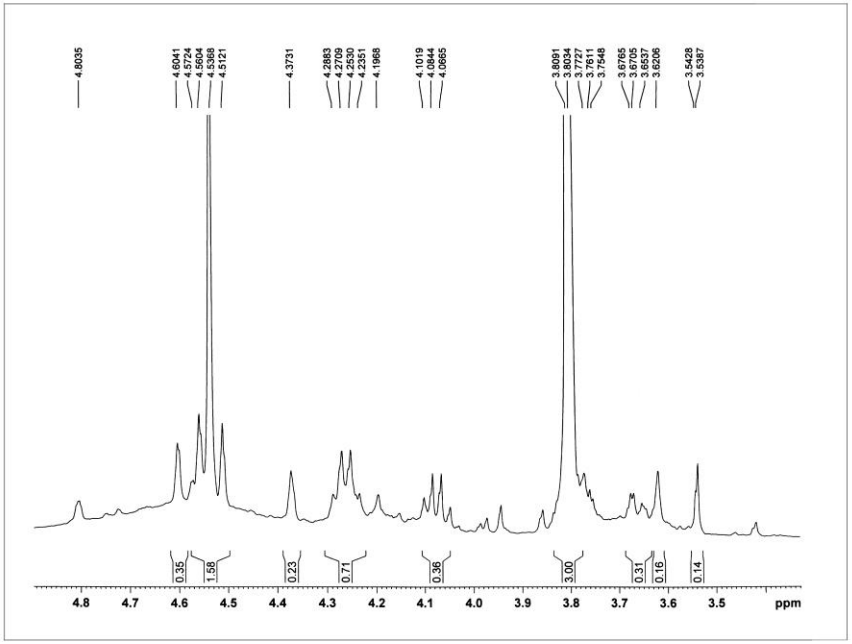
Silica gel 60-120 (0.120mm – 0.250 mm) was used for column chromatography embedded with forosil and Silica Gel G for thin layer chromatography. Florisil has functions for removal of high molecular weight pigments and colouring compounds

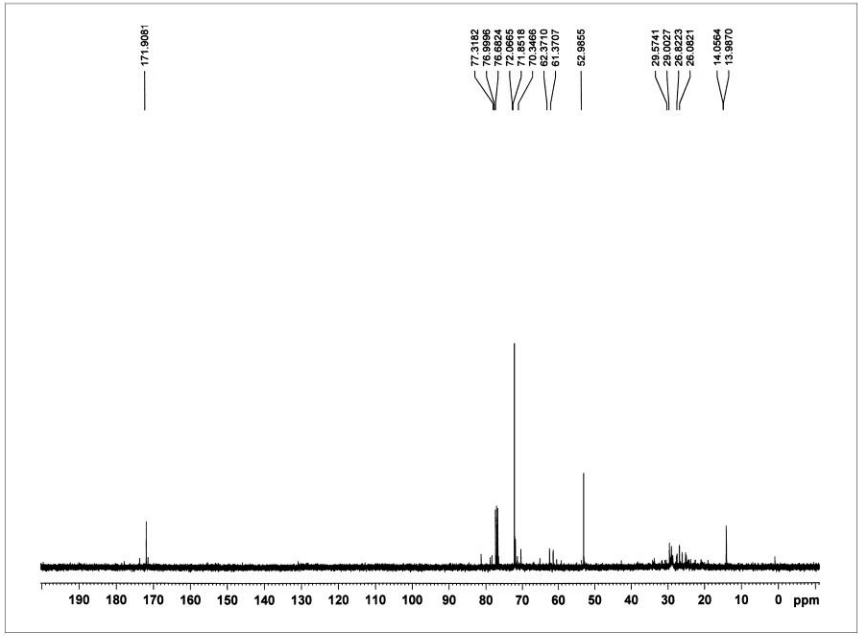
Solvent system used were H, H:E- 8:2; 7:3; 5:5, E:H- 7:3 E, E:M- 9:1; 5:5 and M. Several repetitions were required.(H-Hexane; E- ethyl acetate and M- Methanol)

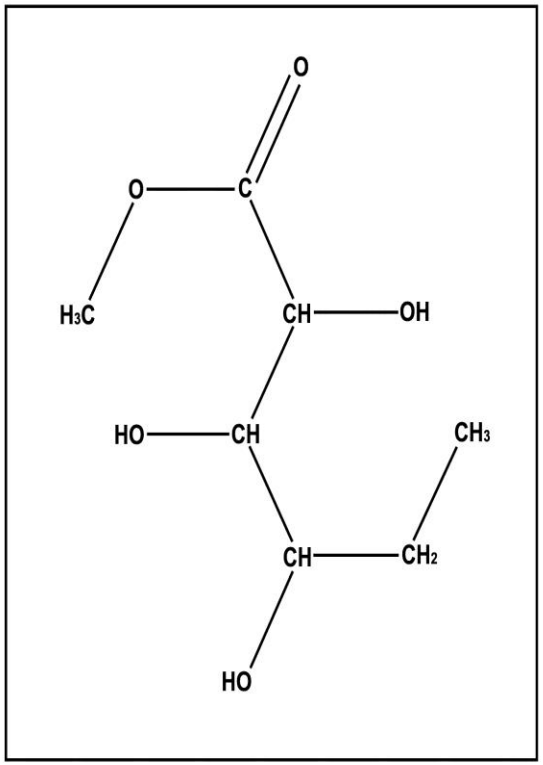
- **Compound:- Ethyl-acetate fraction of *Ipomoea* leaf extract.**
- **Solvent System :–**
  Ethyl acetate : Hexane :: 1:4
- **Spots observed in UV lamp**
- **Rf value :– 0.26**


(0.231) Cm (9:45)
TOF MS ES+
4.56e3
100
%
0
201.03
215.04
187.02
119.03
243.04
179.04
202.03
229.04
133.05
143.03
257.05
171.03
m/z
120 140 160 180 200 220 240 260 280 300 320 340 360 380 400


100.0
%T
3444.42
2928.16
2857.71
1742.54
1444.70
1377.64
1239.73
1132.06
1090.29
976.82
859.03
701.55
600.24
4000.0 3600 3200 2800 2400 2000 1800 1600 1400 1200 1000 800 600 450.0
cm-1
Spectrum Name: 05.sp Accumulations: 16 Resolution: 4.00 cm-1


4.8035
4.6041
4.5724
4.5564
4.5368
4.5121
4.3731
4.2883
4.2709
4.2530
4.2351
4.1968
4.1019
4.0844
4.0665
3.8091
3.8034
3.7777
3.7611
3.7548
3.6765
3.6705
3.6537
3.6206
3.5428
3.5387
0.35
1.58
0.23
0.71
0.36
3.00
0.31
0.16
0.14
4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 ppm


171.9081
77.3182
76.8996
76.6924
72.0665
71.8518
70.3466
62.3710
61.3707
52.9855
29.5741
29.0037
25.8223
25.0821
14.0564
13.9870
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm



Methyl 2,3,4-trihydroxyhexanoate

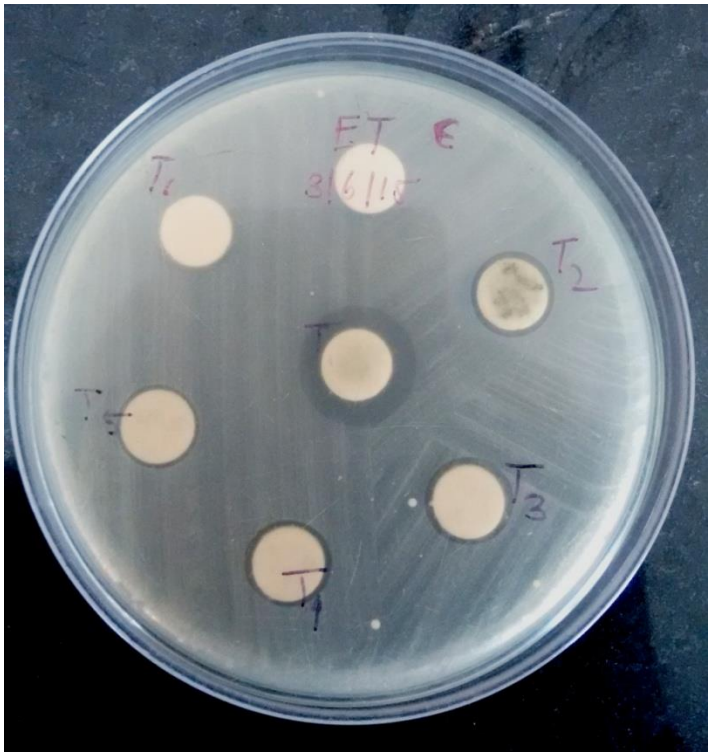
Formula: $C_7H_{14}O_5$

Exact Mass: 178.08

Molecular Weight: 178.18

Antibacterial activity (*Edwardsiella tarda* CGH9)

***Edwardsiella tarda*, causative for emphysematous putrefactive disease of catfish.**

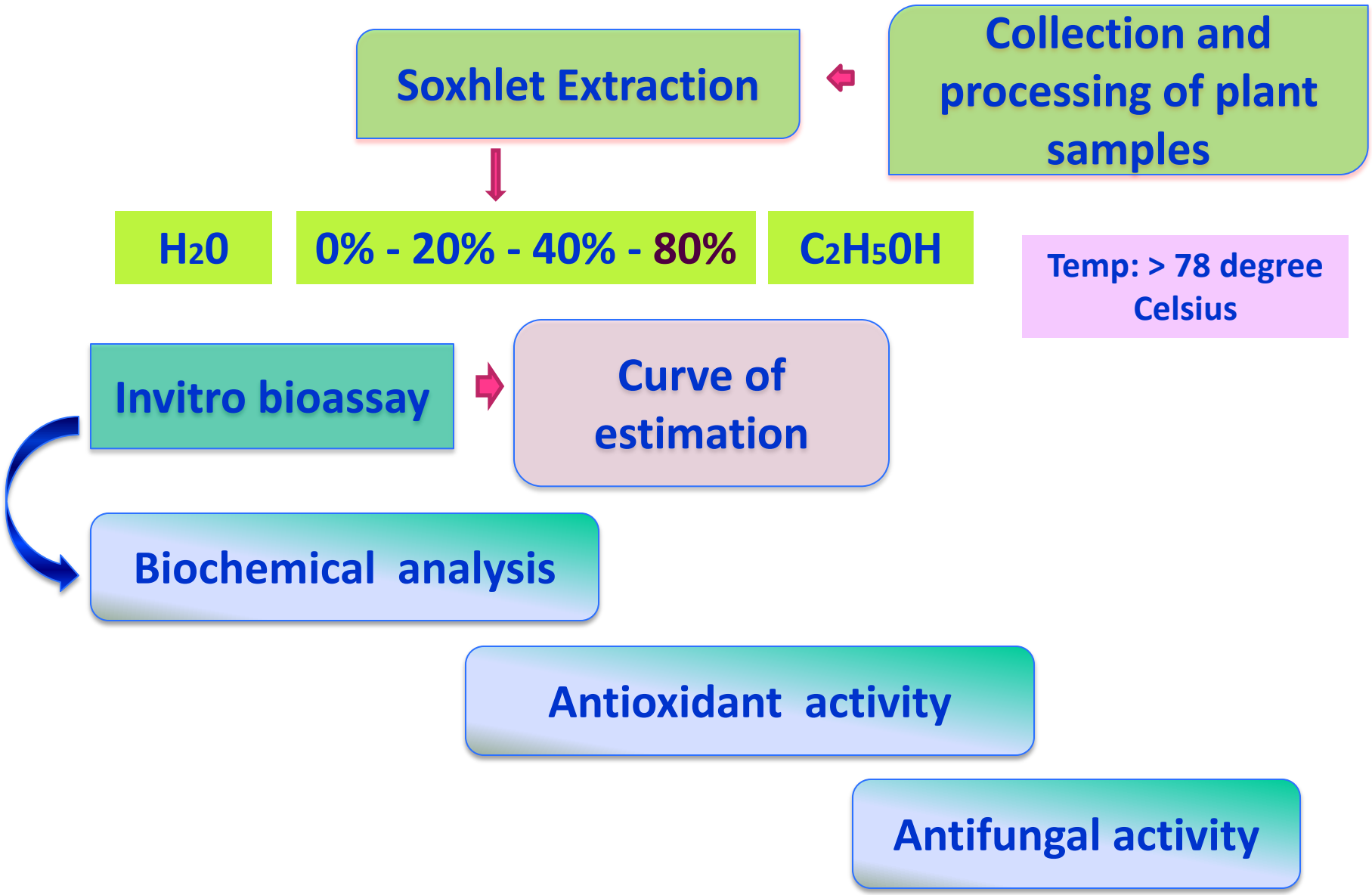


TSB and TSA used for culture

C-control; T6- 1000µg; T2- 500µg; T5-100µg; T3-50µg;T2-10µg and T1-1µg

MIC was found at 1250*µg and MBC at 5000µg*

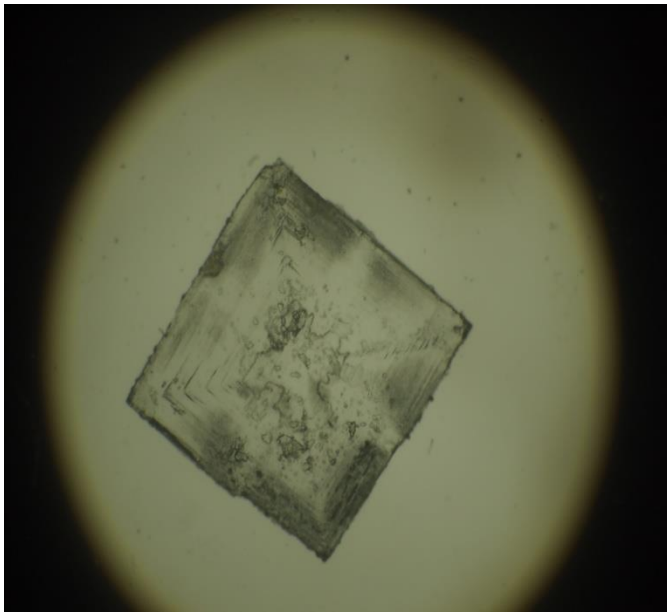
Methodologies for Ethanol extract



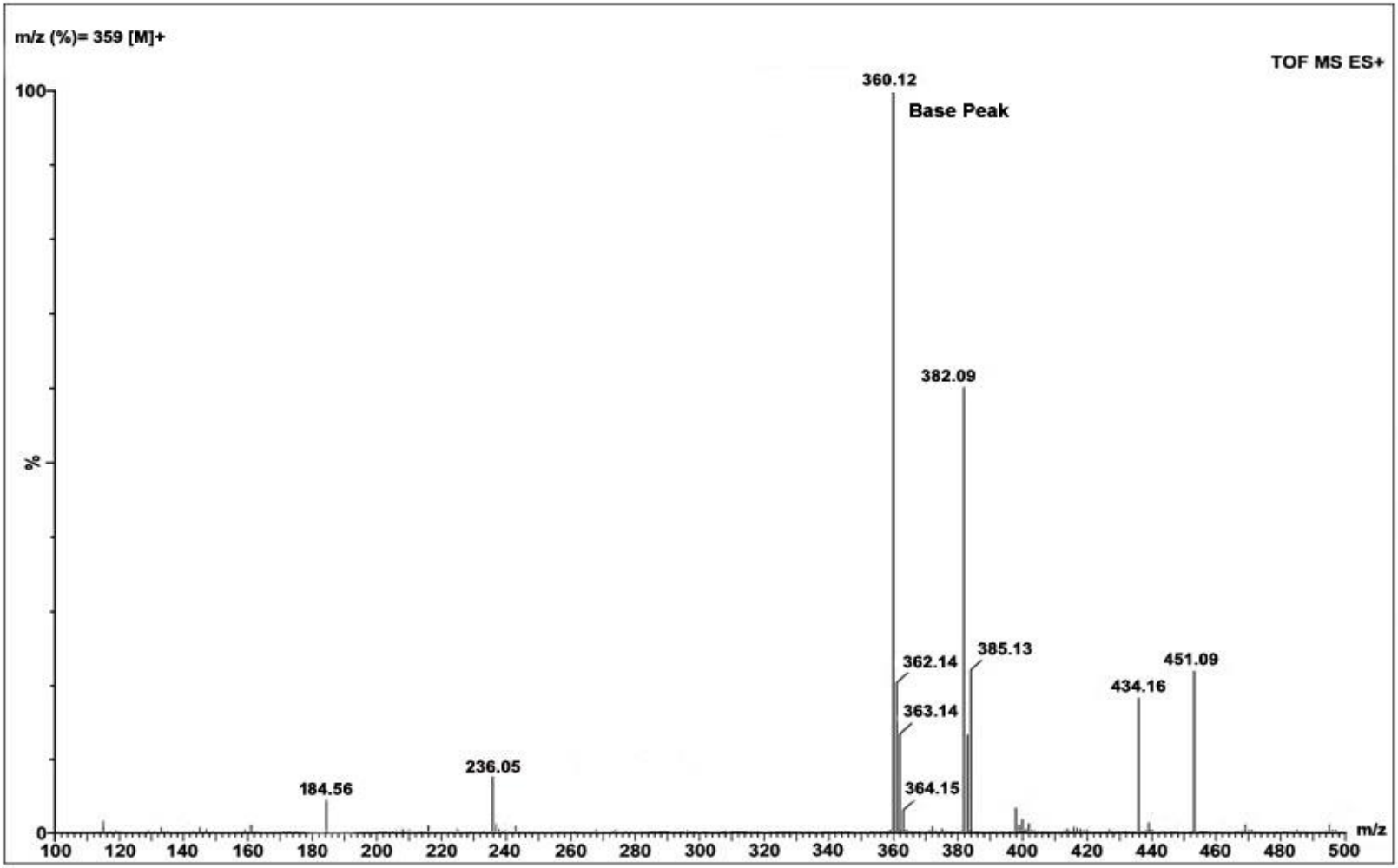
Chromatography

The compound was ideally isolated using solvent system of 1% methanol in ethyl acetate by column chromatography using silica gel 100-230 mesh size and obtained as crystals with chloroform: methanol washes and finally flushed with toluene.

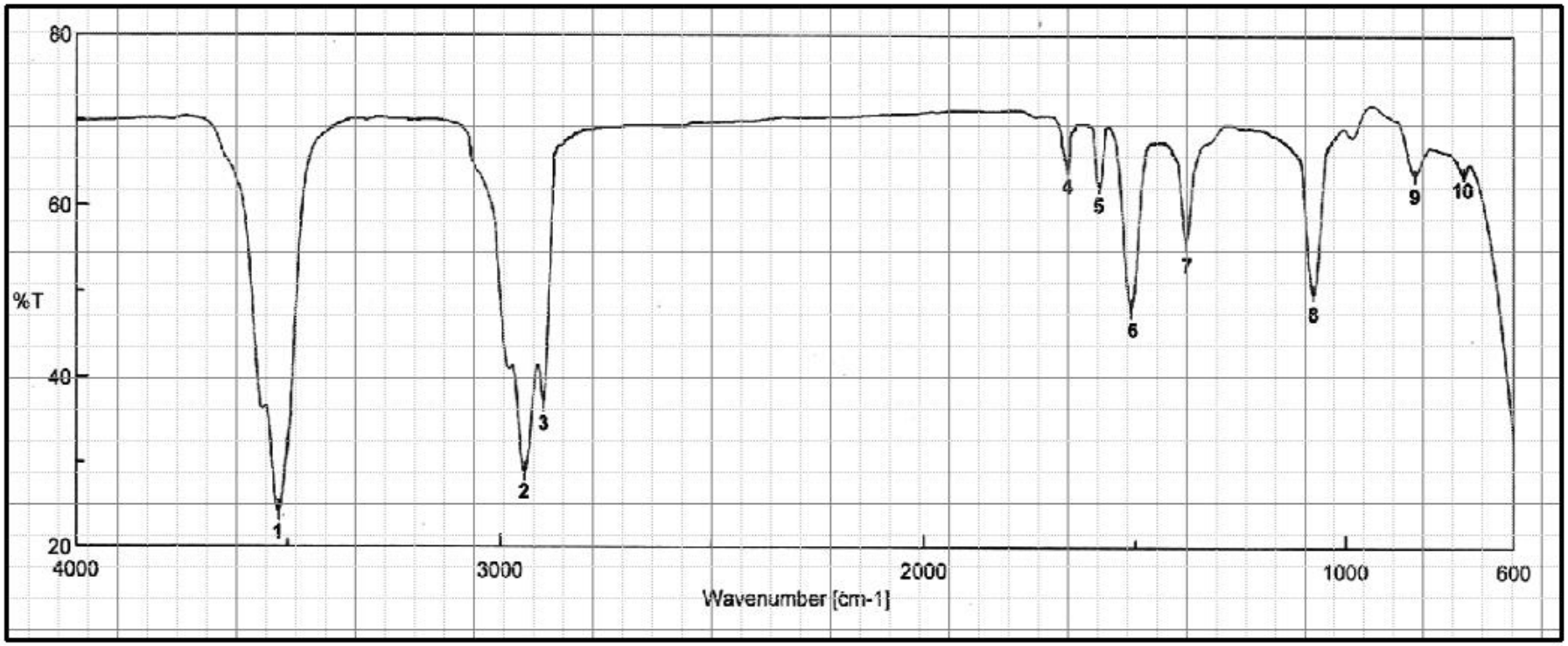
- **Material – Ethanol (80%) fraction of Ipomoea leaf extract**
- **Solvent System – Ethyl acetate: Methanol: Water ::6:1.1:0.1**
- **Spots observed in iodine vapor**
- **Rf value – 0.81**



LC-MS Spectra



IR Spectra



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3642.10	22.0876	2	2965.43	28.1052	3	2928.54	38.9932
4	1665.22	63.2701	5	1600.78	61.7631	6	1554.80	47.1714
7	1360.00	66.3028	8	1080.04	57.1334	9	1076.89	49.4709
10	779.64	62.5325	11	675.16	63.1327			

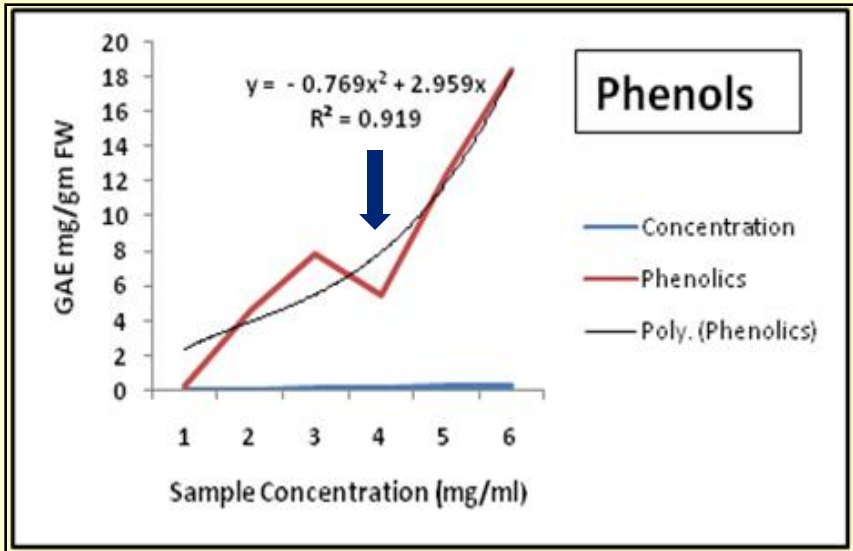
User	SATYA
Division	CHEMISTRY
Company	IICB

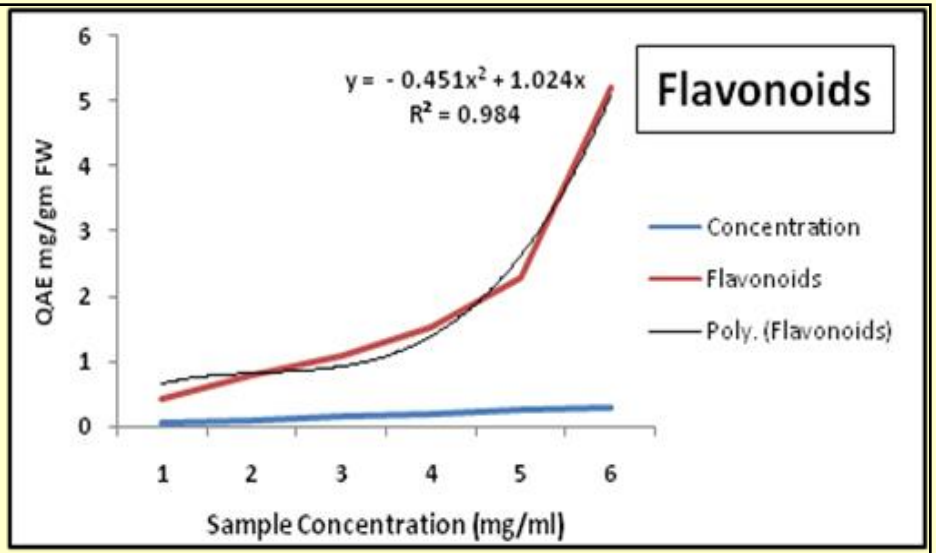
IR Spectra

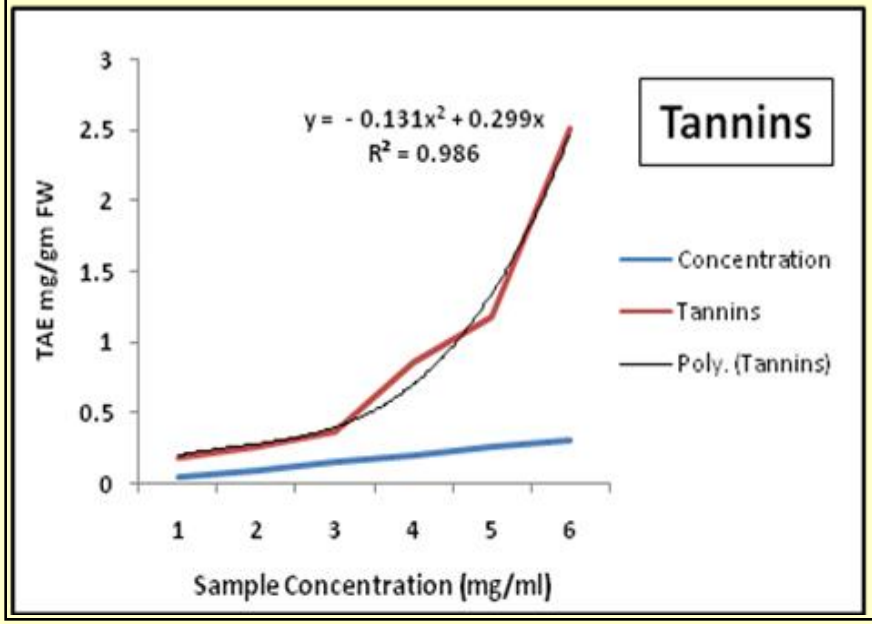
Function Group Prediction

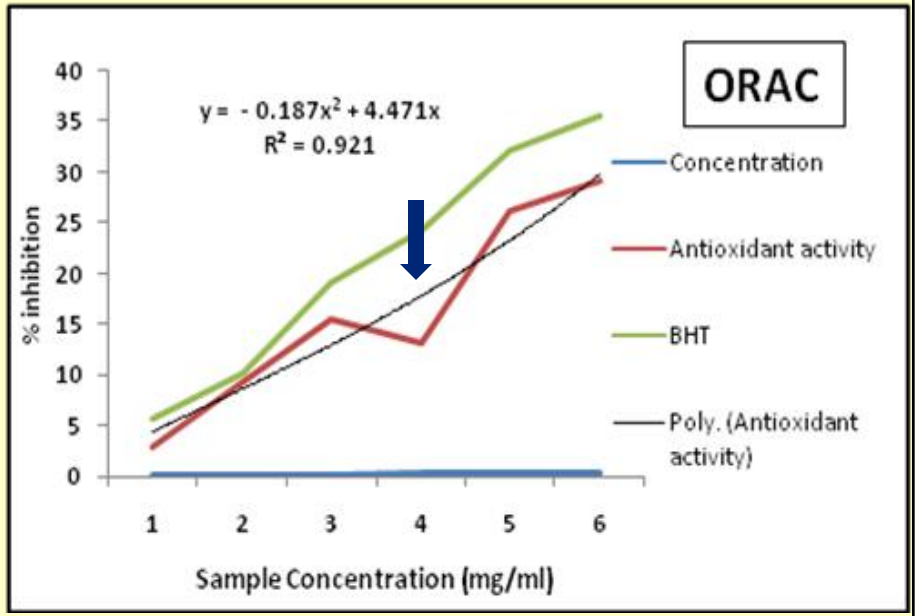
The absorption spectrum of the 80% ethanol fraction shows ten major bands; the band at 3642.10cm-1 corresponds to hydroxyl group usually free hydroxyls. The other dominating bands at 1665.22 cm-1 and 1554.80 cm-1 are those of carbonyls and aromatic compounds. The presence of aromatic compound is further confirmed at 779.64 cm-1 with C-H stretching which if in case of flavonoids, corresponds to the first aromatic ring .The S=O bond usually includes compounds which shows radical trapping antioxidant property and act as antimicrobial, antiparasitic and antitumor agents, a common example of this compound is contained in *Allium sps.*

BIOCHEMICAL ASSAY

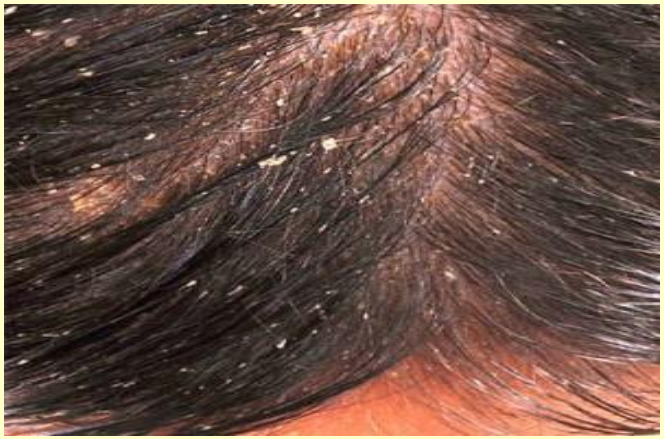


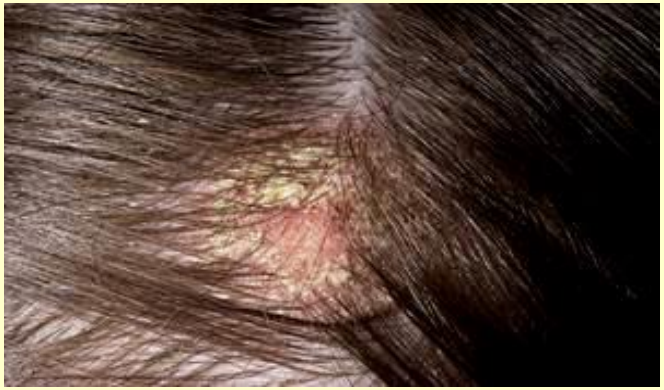


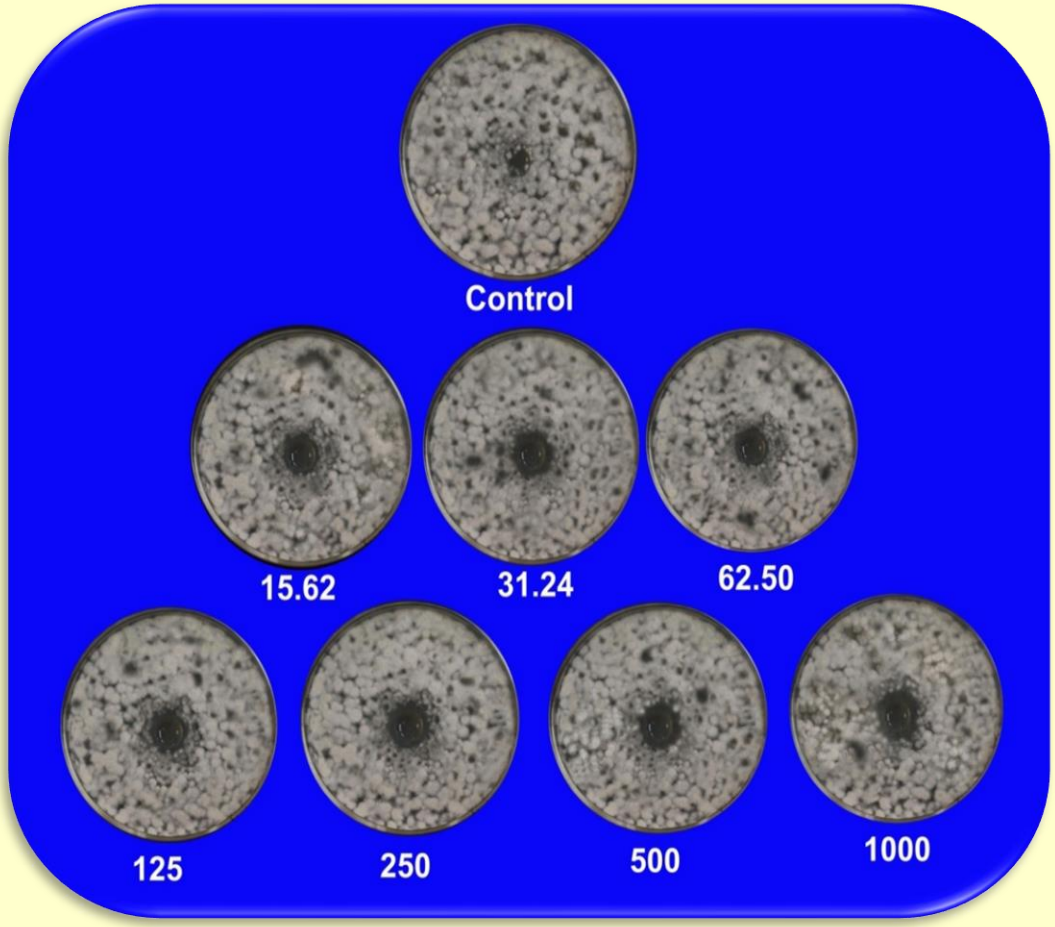




ANTIDERMATITIS ASSAY







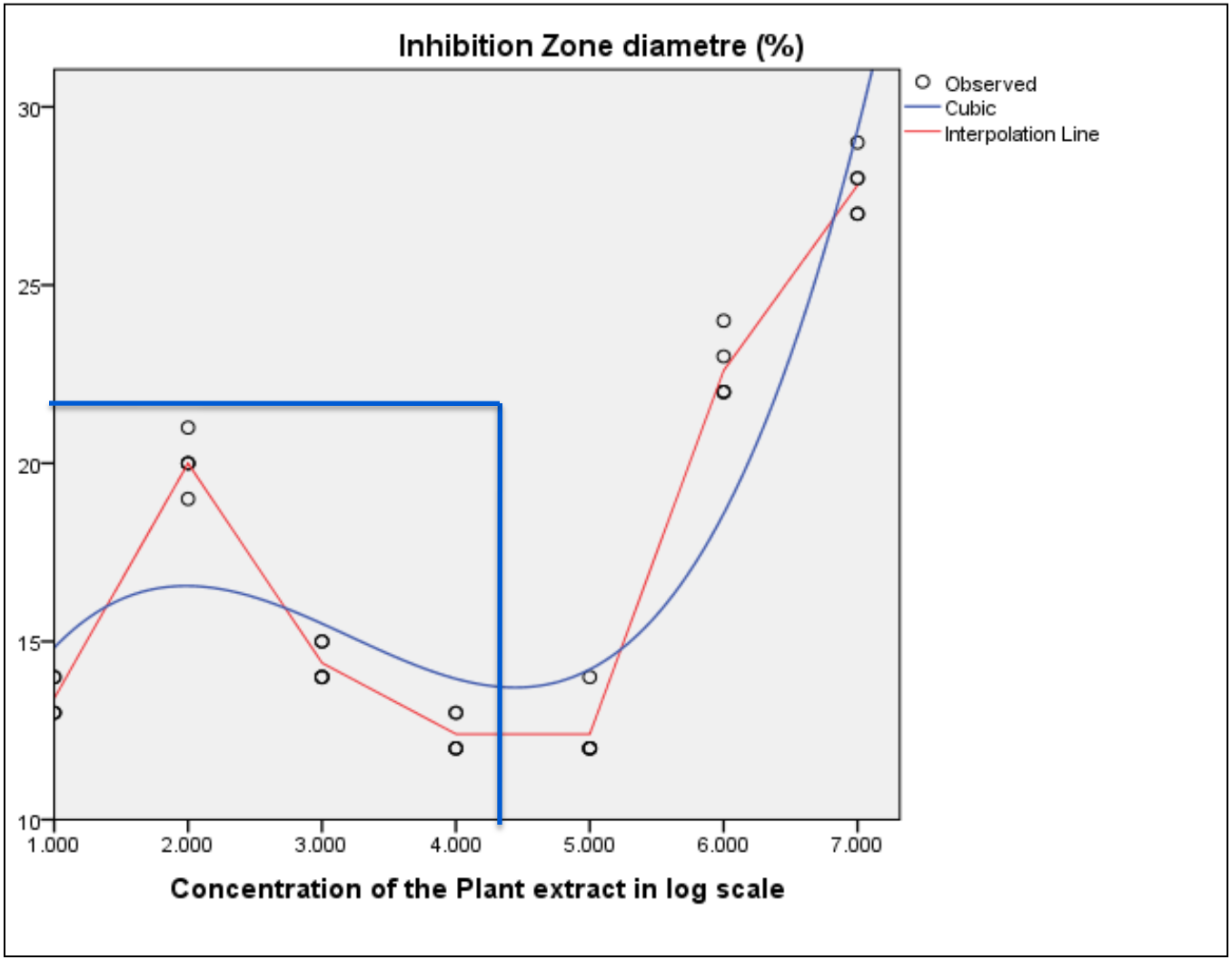
Malassezia globosa

16s contig: *GACGGGAGCT..... TAACCTTCGGGAGG*

Primers: 16S univ, 5’-GAG TTT GAT CCT GGC TCA G-3’ 27f

5’-TAC GGT TAC CTT GTT ACG AC-3’ 1492r

NON-MONOTONIC CURVE



WAY FORWARD

- Non-Linear concentration dependant degradation kinetics of allelochemicals.
- Studying the entire pathogenicity of the compounds
- Comparing it with market based standards.
- The strategies to utilize *Ipomoea* extract to eradicate algal bloom.

THANK YOU


Rinkle
HOT AIR
OFF
COOL
WARM
HOT
FITTED WITH AUTOMATIC
THERMAL CUT OFF

Phenols

The total phenolic content was determined following Folin-Ciocalteu method using 0.1ml of the extract with a concentration range of 0.05-0.3 mg/ml of the leaf leachate. The extracts were mixed with Folin-Ciocalteu reagent and Sodium carbonate (Na_2CO_3) following incubation for 30 mins at room temperature. The change of colour was measured in spectrophotometer with absorbance reading at 765 nm. Gallic acid in the same concentration as the sample was used as positive control. The total phenol content was expressed as Gallic acid equivalents (GAE) in milligram per gram of dry material using the calibration curve, where X was the absorbance and Y was the Gallic acid equivalent (mg/g).

Flavonoids

Flavonoid estimation was carried out following the method of Jia *et al.*,[9].The preferred concentration range for the leaf leachates were 0.2-1.2 mg/ml with 0.1ml of the extract. Later the extract was added with 1.2 ml distilled water, 0.12 ml of 5% Sodium nitrite ($NaNO_2$) with uniform intermixing. Following incubation for 5 mins at 25 º C temperatures, 0.12 ml of 10% $AlCl_3$ solution was added and mixed thoroughly. Then the tubes were further incubated at room temperature for 5 minutes and added with 0.8 ml of 1 mM Sodium hydroxide (NaOH) solution and 1.16 ml of distilled water. The absorbance was measured at 510 nm. Methodically, quercetin in the same concentration as the sample was used as positive control. Total flavonoids content was calculated as Quercetin (mg/g) using the calibration curve, where X was the absorbance and Y was the Quercetin equivalent (mg/g).

Tannins

Europeenne Commission (2000) reference method was used to determine the total tannins content using tannic acid as standard curve. Briefly, 200 µL of extracts of $0.05 - 0.3$ mg/ml wsa mixed with 200 µL of ferric ammonium citrate (0.35%) prepared freshly and 200 µL of ammoniac (0.8%). The absorbance of the mixture was measured at 525 nm. The results were expressed as mg of Tannic Acid Equivalent (TAE) per gram of extracts or fractions.

Antioxidant assay

DPPH radical scavenging activity: The free radical scavenging activity of extracts and fractions for the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described by **[11]**. Freshly prepared DPPH solution (25 mg/L) in methanol was prepared and 3.9 ml of this solution was mixed with 0.1 ml of extract in methanol containing different concentration range (0.05-0.3 mg/ml conc.) of the extract. 30 minutes later, the absorbance was measured at 517 nm using Spectrophotometer. Butylated Hydroxy Toluene (BHT) in the same concentration as the sample was used as positive control. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH radical scavenging activity (%) = {Ac-At/Ac} x 100

Where Ac is the absorbance of the blank reaction and At is the absorbance in presence of the sample of the extracts.