MATERIALS AND METHODS

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1.Ichinocarpus frutescens

a)PLANT HISTORY:

Ichinocarpus frutescens, Ait.Hort.Kew.ed 2, v, 2(1811)P 69

Family-Apocynaceae

Vernacular name-Kali-duddhi

Distribution:

Distributed throughout India in plains and lower hills upto, 2000m. A much branched extensively climbing, rusty villous evergreen, lacticiferous woody, climber, leaves simple, opposite, elliptic, oblong to broadly lanceolate, acute or accuminate main nerves 5-7 pairs, flowers greenish white, fragrant, numerous in axillary or terminal panicles of cymose clusters, fruits straight or slightly curved, seeds black, flowers Nov-Dec.

b) PLANT MATERIAL:

Ichnocarpus frutescens is an evergreen twining shrub. Its roots are reported to posses various pharmacological properties and used in disease control.

The seeds of *I.frutescens* were collected from Koyananagar,Dist-Satara.

c) EXTRACTION:

The collected seeds of *I.frutescens* were air dried at room temperature and ground to a fine powder. Then about 500gm of fine powder was soaked in ethanol for about 7-8 days in a glass jar. After 7-8 days the extract was filtered through four folds of musclin cloth and the filterate obtained was evaporated by rotary evaporation to get brown coloured residue. This residue was used for further seperation method.

d) SEPARATION METHOD :

There are different techniques of separation like Thin layer chromatography, Gas chromatography, HPLC, Column chromatography etc. Out of these techniques we have followed Thin layer chromatography and Column chromatography.

Thin layer chromatography:-

i) PREPARATION OF TLC PLATES:

The plates were prepared by dipping method (Peifer & Microchim, 1962)[42]. A suspension Of 35gm Silica Gel G and 100ml chloroform was shaken vigorously for 3 min in a stoppered conical flask.A pair of thoroughly washed plates were held together, dipped into the suspension and then slowely withdrawn. Then the plates were separated and solvent was evaporated, and the steam was passed over the plates, so that calcium sulphate sets properly.

ii)APPLICATION OF SAMPLE:

The sample solution (extract in alcohol) were applied on the chromatographic plates with the help of capillary tubes. The samples were then air dried.

iii)SOLVENT SYSTEM:

Then the plates were placed in a developing chamber. The chamber was perfectly closed with a glass lid. It was kept for about 30 to 35 min. After complete development TLC plates were taken out and air dried.The solvent system used for development was 50% methanol and 50%chloroform.

iv)DETECTION OF SPOT:

For the detection of spots iodine was used as a visualising reagent. The chromatographic plates were put into iodine chamber for few minutes. The yellow brown spots were developed when they came in contact with iodine vapours.

Rf value was calculated.

$$Rf = \frac{\text{Distance travelled by solute front}}{\text{Distance travelled by solvent front}}$$

I) Fraction I: (Yellowish brown colour spot)

$$Rf = \frac{9.5}{11.9}$$

Rf= o.79

II) Fraction II: (Yellowish brown colour spot)

$$Rf = \frac{11.4}{11.6}$$

Rf = o.98

Separation of Fraction with Column Chromatography

a) PREPARATION OF COLUMN:

The adsorbent medium used was the column filled with silica gel which was previously activated. A cotton swab was fitted at the bottom of column (Burette). Then the adsorbent silica gel was added to the column. The height of the adsorbent was about 9-10 cm. An another cotton swab was also fitted at the top of column.

b) SEPARATION OF COMPONENTS : (FRACTIONS)

The extracts were added in the column which was previously wetted with the solvent and allowed to run in downward direction. The eluent benzene and methanol was added above the cotton swab.The fractions separated by column were collected in beakers as fraction I and II. These fractions I and II were used for further testing i.e.antifungal, antibacterial and antifeedent activity.

e) PHYSICAL DATA:

a) Fraction-I (Benzene fraction)

i) Colour of compound:- Pale yellow

ii) Nature of compound:- Semisolid

iii) M.P.(observed) : 37^0C

b) Fraction-II (Methanol fraction)

Colour Of compound:- Brown

Nature of compound:-Semisolid

M.P.(observed) : 70^0 C

SPECTRAL DATA:

Fraction-I:

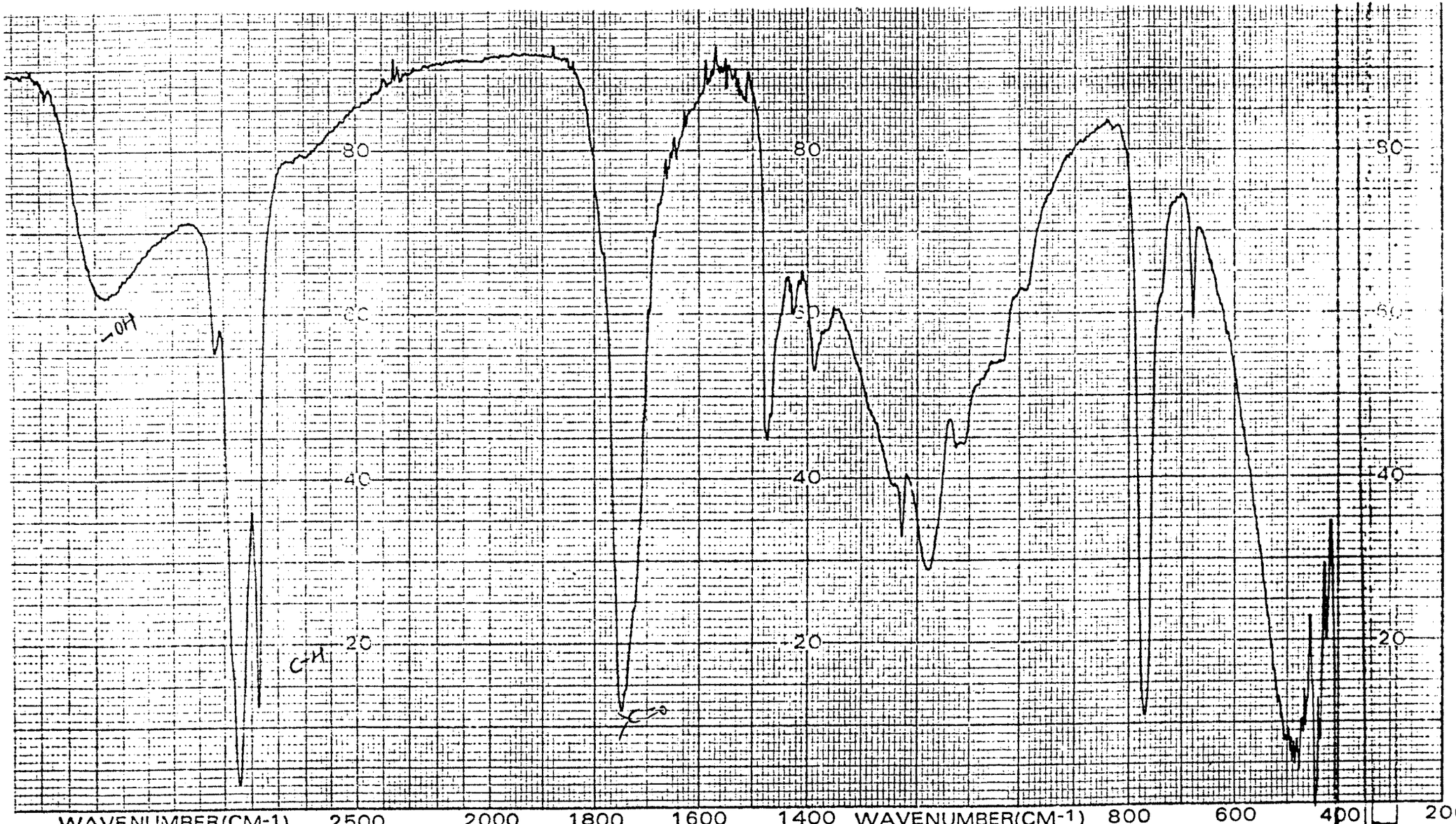
1) IR(Nujol): 3500 cm^{-1} -alcoholic -OH group

1750 cm-1-acetate >C=O group.

(Fig. No.1)

2) UV(Ethanol): λmax 260nm may be congugated >C=O.

(Fig. No.2)



ABSCISSA	ORDINATE	SCAN TIME	REP. SCAN / SINGLE BEAM
EXPANSION	EXPANSION	MULTIPLIER	TIME DRIVE
PRESSION	% T / ABS	SLIT PROGRAM	OPERATOR / DATE 26.8.86
SAMPLE #24	REMARKS	SOLVENT Neat	CELL PATH
ORIGIN A.B. Ajgaonkar	Fig. No. 1 - IR spectrum	CONCENTRATION	REFERNCE

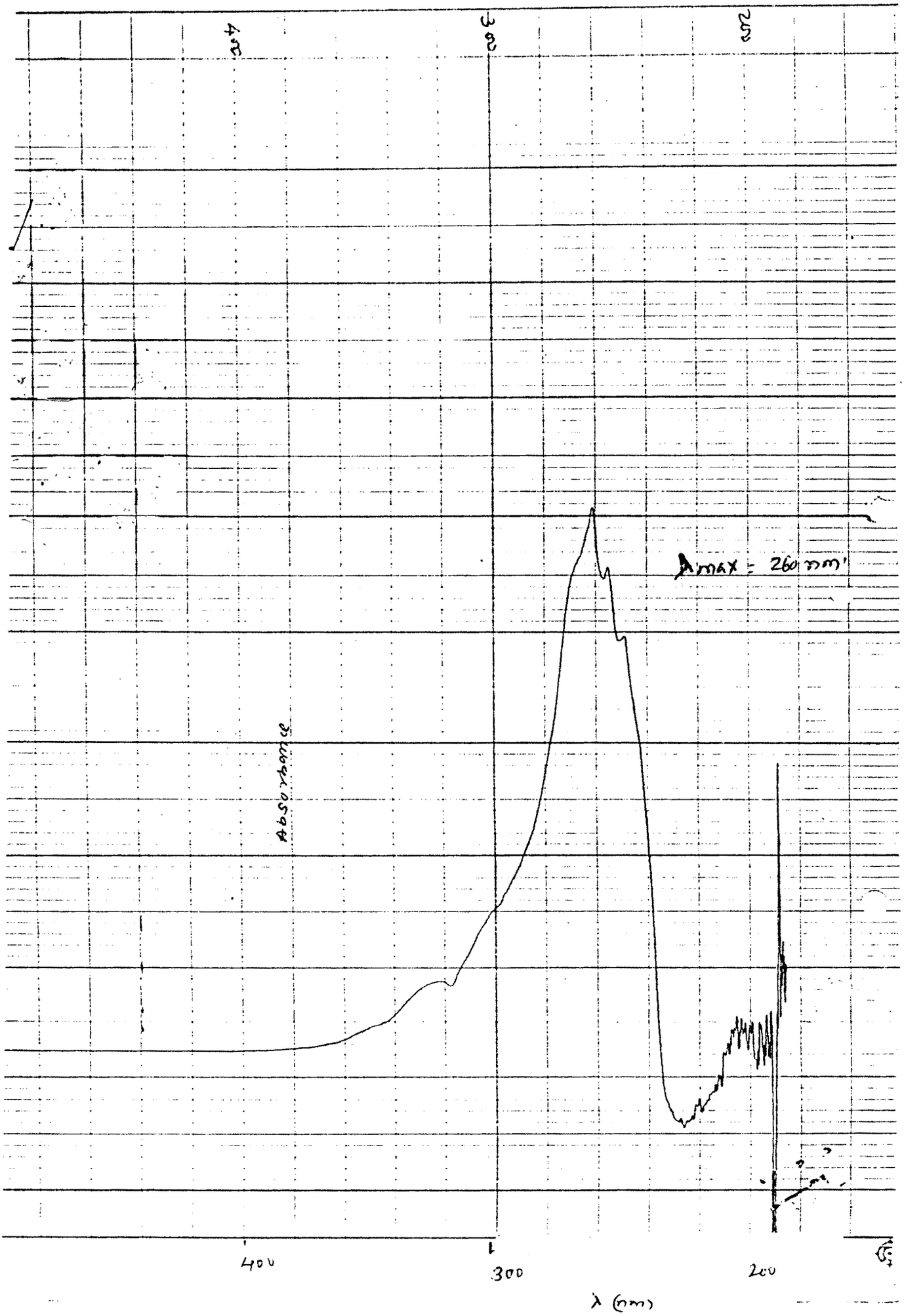


Fig No - 2

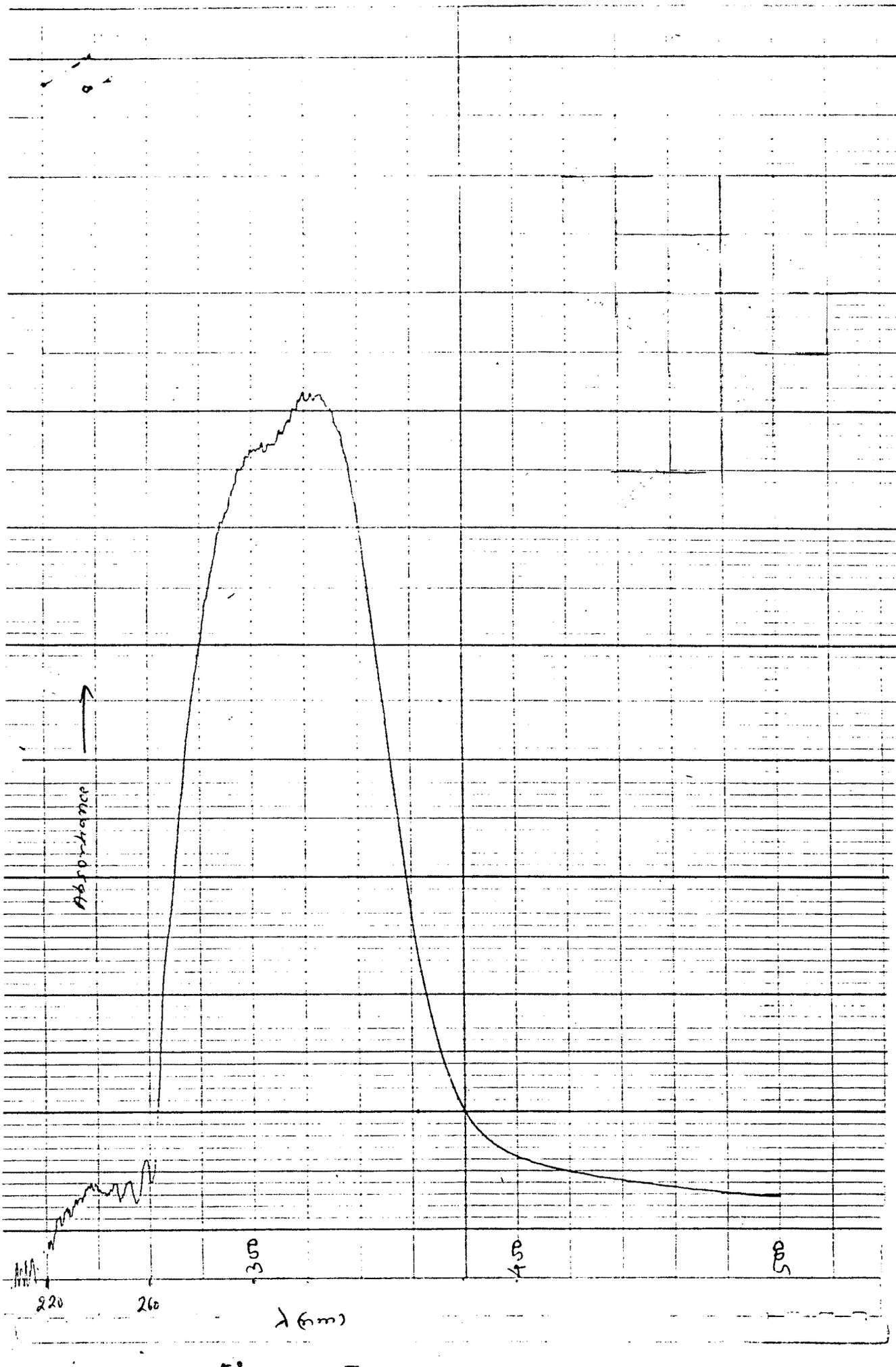


Fig. NO.- 3

Fraction II:

1)UV(Ethanol): λmax 260 nm may be congugated >C=O

(Fig.No.3)

2.*Homononia riparia* :

a)PLANT HISTORY:

Homononia riparia Lour Fl.Cochinch.

Family-Euphorbiaceae

Vernacular name-Sherni

Distribution-

Distributed throughout India,at lower elevation on the banks of rivers and streams especially among rocks.

Rigid evergreen willow like dioecious shrub with dark grey or brown bark and greyish brown moderately hardwood and pubesent young parts, leaves linear or linear oblong, entire or more or less serrulate towards the apex, glabrous above and glandular scaly beneath, main nerves 10-30,

pairs flowers sessile in axillary elongated, pubesent spikes, stamens in male, flowers papillose,fruits globose capsules,seeds yellowish brown,smooth, slightly angular on the inner surface and rounded on the back.

b) PLANT MATERIAL:

Homononia riparia,is an evergreen shrub.Its leaves,fruits and roots posses various pharmacological properties.

The seeds of *Homononia riparia* were collected from Helwak,Dist-Satara (Western ghat).

c) EXTRACTION AND SEPARATION:

The extraction of seed material and separation of different fraction was carried out as discussed in *Ichinocarpus frutescens* using thin layer chromatography and column chromatography.

Rf value calculated from TLC were as follows

1)Fraction-I: Brown coloured spot

$$Rf = \frac{\text{Dist.travelled by solute front}}{\text{Dist.travelled by solvent front}}$$

$$Rf = \frac{8.5}{11.1}$$

$$Rf = 0.76$$

2)Fraction-II: Brown coloured spot

$$Rf = \frac{9}{10}$$

$$Rf = 0.75$$

d) PHYSICAL DATA:

a) Fraction-I: (petroleum ether fraction)

i) Colour of compound-Brown

ii) Nature of compound-Semisolid

iii) Melting point(observed): 39^{0}C

b) Fraction-II: (Benzene fraction)

Colour of compound-Yellowish brown

Nature of compound-Semisolid

Melting point(observed): 45^{0}C

3) *Laportea interrupta:*

a) PLANT HISTORY:

Laportea interrupta (L) chew.Gard,Bull Singapore 19,200,1965,

Urtica interrupta L.Sp.Pl.985,1753,Weight Icont 692 1840,

Fleurya interupta (L).Gaud in Uranic vay Bot-12 497t.8-830,Wight Icont 1975,1853.

Family : Urticeae

Vernacular name : Khatkhuttil

Distribution :

Distributed throughout India, common along old walls of houses,in shades.

It is 30-60 cm high herb,branchlets with scattered stinging hairs,leaves alternate,broadly ovate, sparsely hairy,base acute,margin coarsely serrate, apex shortely accuminate,flowers monoecious greenish white, flowers and fruits in August to December.

b)PLANT MATERIAL:

The seeds of *Laportea interrupta* were collected from Koyananagar,Dist.-Satara.

c)EXTRACTION AND SEPARATION:

The extraction of seed material and separation of different fraction was carried out as discussed in *Ichinocarpus frutescens* using

Thin layer chromatography and Column chromatographic method.

Rf value calculated from TLC was as follows,

1)Fraction I:-Brown coloured spot

$$Rf = \frac{\text{Dist. travelled by solute front}}{\text{Dist.travelled by solvent front}}$$

$$Rf = \frac{10.3}{11.4}$$

Rf= 0.90

d)PHYSICAL DATA:

a)Fraction-I: Benzene fraction

i)Colour of compound-Brown

ii)Nature of compound-Thick liquid

iii)Boiling point-Having B.P. more than 350^{o}C

4) *Vernonia anthelmintica* Willd

Centratherum anthelminticum (Linn) O.Ktze.

Family : Asteraceae

Vernacular name : Kalijira,Somaraj

Distribution:

It is found in waste lands near village throughout India.

a) PLANT HISTORY:

It is a tall robust,stout,erect annual and 60 to 90 cm in height with pubescent branches. Leaves are long, lanceolate or ovate-lanceolate, acute, coarsly serrate, mambranous, more or less pubescent on both sides, base tapering into petiole, flowers purplish in subcorymbose head, outer involucral bracts linear and hairy, green with purplish obtuse tips, innermost bract longest, fruits achenes, oblong, cylindric, 10 ribbe pubescent, pappus reddish, inner pappus long, outer short.

b) PLANT MATERIAL:

The seeds of *Vernonia anthelmintica* were Collected from Helwak Dist-Satara (western ghat).

c)EXTRACTION AND SEPARATION:

The extraction of seed material and separation of different fraction was carried out as discussed in *Ichinocarpus frutescens*. The separation was carried out using thin layer chromatography and Column chromatography.

The Rf values calculated from TLC were as follows

1)Fraction-I: (Yellow colour spot)

$$Rf = \frac{\text{Dist.travelled by solute front}}{\text{Dist.travelled by solvent front}}$$

Rf = 9/11

Rf = 0.81

2) Fraction -II: (Yellow colour spot)

$$Rf = \frac{9}{9.8}$$

$$Rf = 0.91$$

d) PHYSICAL DATA:

A) Fraction -I: (Benzene fraction)

i) Colour of compound-Yellowish brown

ii) Nature of compound- Semisolid

iii) M.P. (observed) : 40° C

B) Fraction -II: (Methanol fraction)

i) Colour of compound-Yellow

ii) Nature of compound-Semisolid

iii) M.P. (observed) : 38°C

e) SPECTRAL DATA:

Fraction-I

1) IR (Nujol): λmax 3400-3000cm^{-1}-alcoholic-OH

1735cm^{-1}-Acetate or Ester >C=O

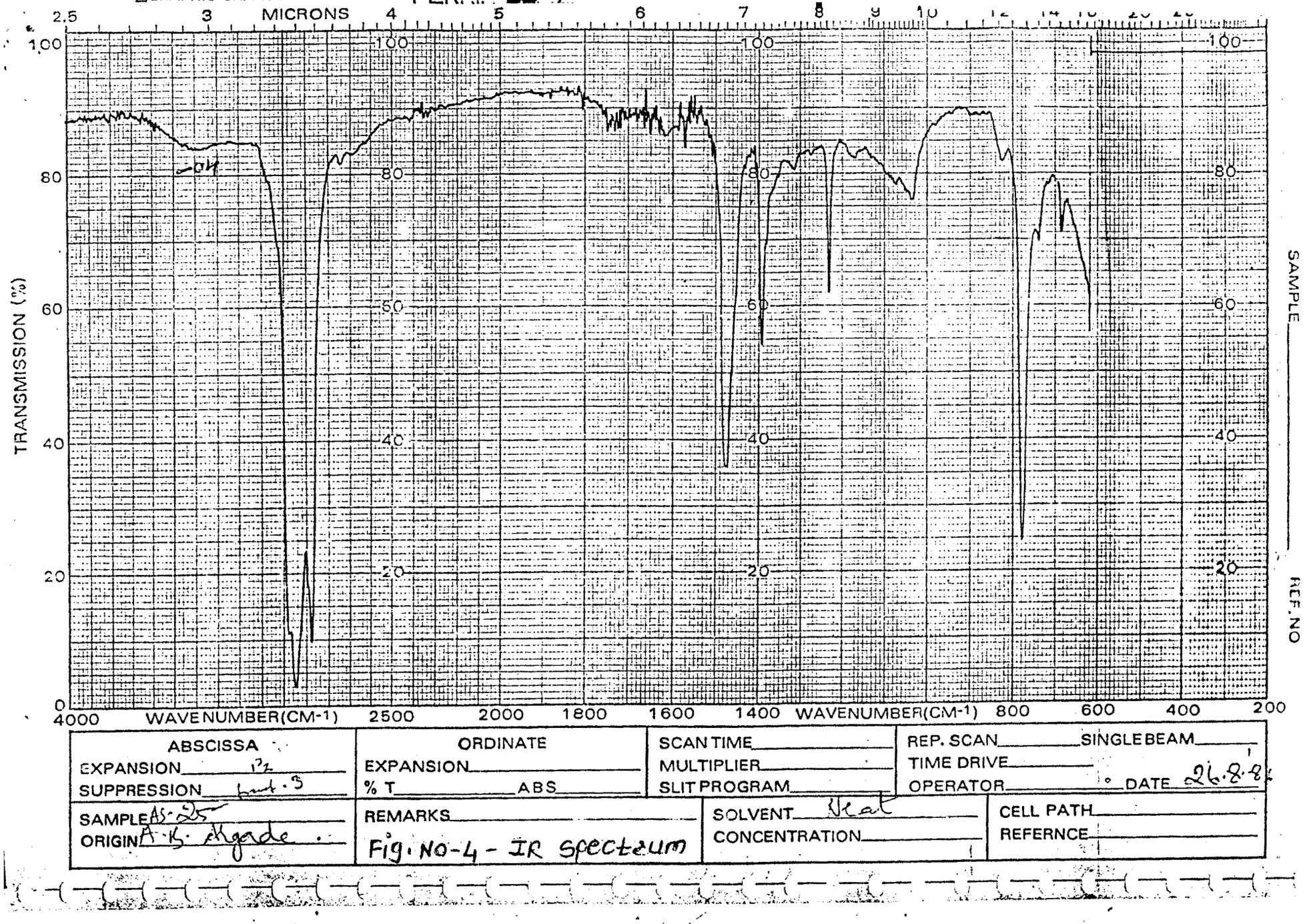
(Fig.No.4)

2) Mass spectra: m/e value-496(2%), 467(3%),
439(3%),367(7%),339(15%),
313(25%),283(10%),262(50%),
239(50%),227(42%),213(25%),
193(24%),183(50%),171(84%),
135(68%),123(58%),109(87%),
95(62%),81(45%),69(55%),
57(42%),43(19%).
(Fig. No.5)

Fraction-II:

1) IR (Nujol): 3000-3500cm^{-1}-alcoholic OH group.

(Fig. No. 6)



ABSCISSA	ORDINATE	SCAN TIME	REP. SCAN SINGLE BEAM
EXPANSION 1½	EXPANSION	MULTIPLIER	TIME DRIVE
SUPPRESSION Int. 3	% T ABS	SLIT PROGRAM	OPERATOR DATE 26.8.84

SAMPLE AS-25	REMARKS	SOLVENT Neat	CELL PATH
ORIGIN A.K. Agade.	Fig. No-4 - IR Spectrum	CONCENTRATION	REFERNCE

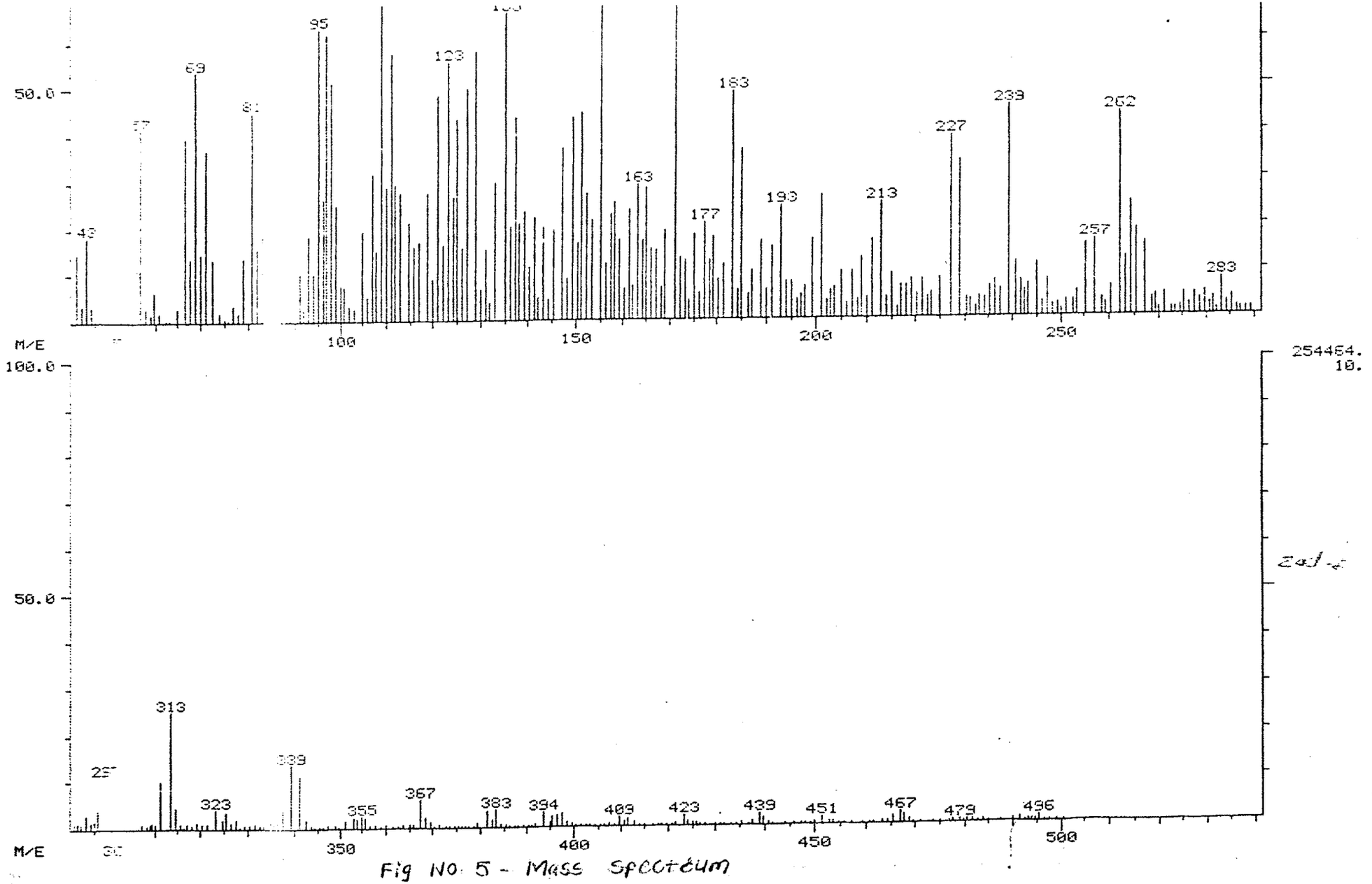
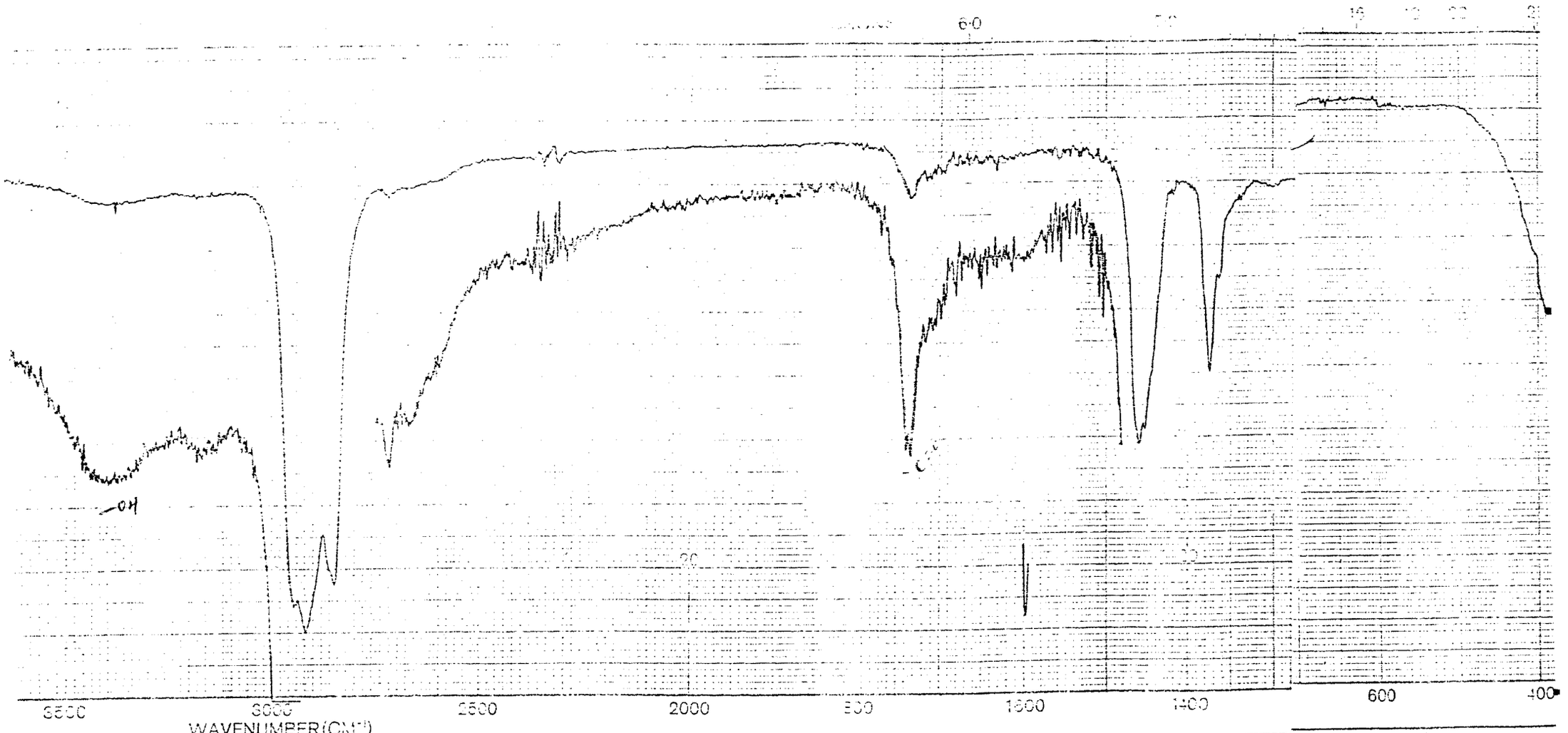


Fig No. 5 - Mass Spectrum



SOLVENT Nujol
CONCENTRATION
CELL PATH
REFERENCE Poly styrene 1601.4

REMARKS
Fig No·6 - IR SPECTRUM

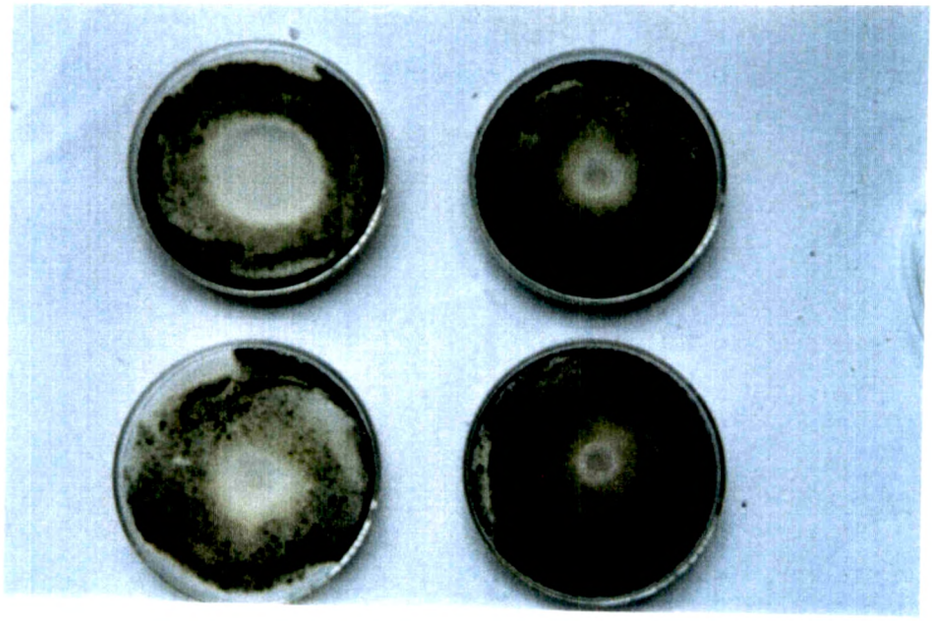
SLIT PROGRAM 3
SCAN TIME 6 cm/min
MULTIPLIER 1
TIME CONSTANT 1
DATE 12-2

PERKIN
CHART No
REF No. IR-

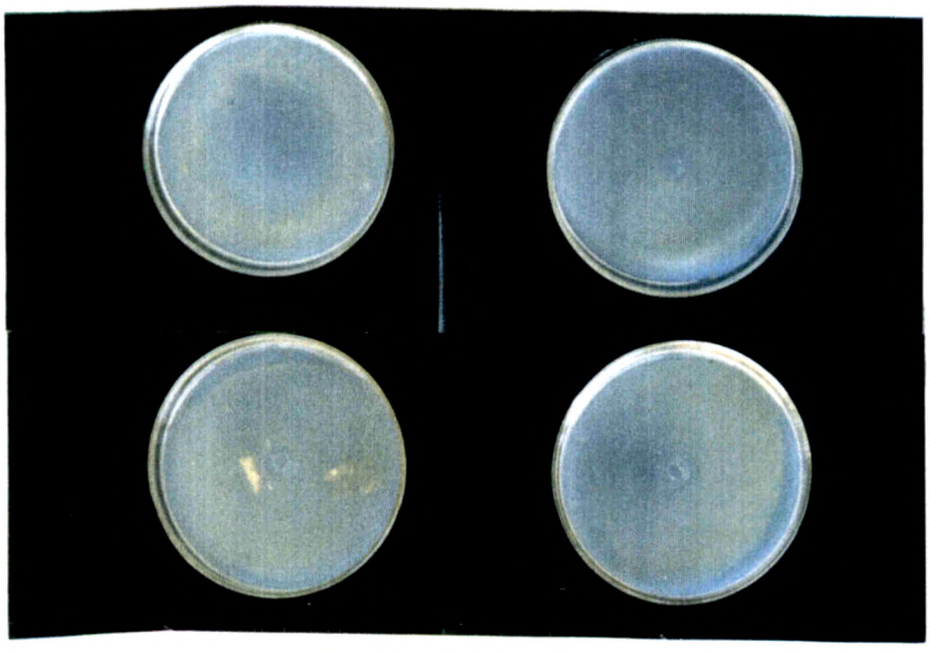
Solanum surattense plant

Flowers, Fruits and leaves of *Solanum surattense*

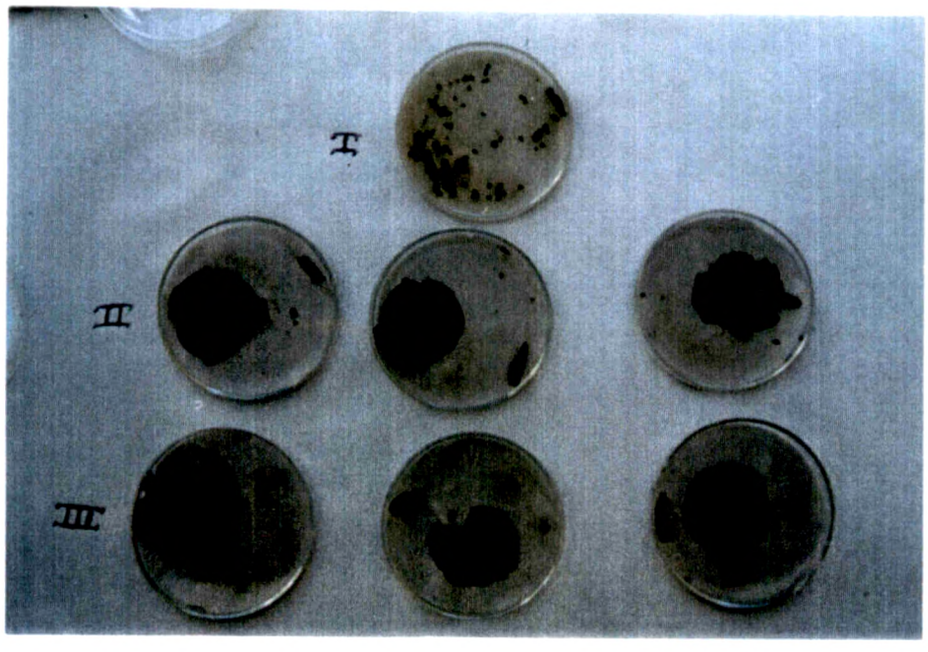
Homonoia riparia plant



Antifungal activity of plant extract
against *Aspergillus spp*
Standard 500 ppm
250 ppm 100 ppm



Antibacterial activity of plant extra
against *Xanthomonas citri*
Standard 500 ppm
250ppm 100 ppm



Antifeedant activity of plant extrac
against Red hairy catterpillar
I-control
II-*Laportea interrupta*
500 ppm, 250 ppm, 100 ppm
III-*Solanum surettense*
500 ppm ,250 ppm, 100 ppm

5) *Solanum surettense* Burm f.

Solanum virginianum

Solanum xanthocarpum Schard & Wendl.

a) PLANT HISTORY:

Solanum surattense Burm F.Fl.Ind.-1768.

Solanum xanthocarpum Schard & Wendl.Sert. Hannov 1:8:t.2 1765.

Family-Solanaceae

Vernacular name-Kantakari

Distribution:

It is distributed throughout India, in dry situation as a weed on roadsides and waste lands. A prickly much branched herb,usually spreading or diffuse,young branches densely covered with minute star shaped hairs, prickles yellow, shining about 1.5 cm long leaves upto 10cm long,their midribs and other nerves with sharp

yellow prickles,flower purple,about 2cm long,few together in small bunches opposite the leaves, fruits 1.5 to 2 cm round,yellow or pale with green veins.

b) PLANT MATERIAL:

The fruits of *Solanum surettense* were collected from Koyananagar Dist.: Satara.

c) EXTRACTION AND SEPARATION:

The extraction of fruit material and separation was carried out as discussed in *I.frutescens.* The separation was carried out using TLC and Column chromatography. Rf value calculated from TLC was given as -

1) Fraction -I: (Brown colour spot)

$$Rf = \frac{\text{Dist.travelled by solute front}}{\text{Dist.travelled by solvent front}}$$

$$Rf = \frac{9.5}{11}$$

Rf= 0.86

d) PHYSICAL DATA:

A) Fraction-I : (Benzene fraction)

i) Colour of compound-Yellowish brown

ii) Nature of compound-Semisolid

iii) M.P.(observsd): $45^{0}C$

ANTIMICROBIAL ACTIVITY

Many plant reported to posses antimicrobial activities against various fungi and bacteria. A variety of chemicals having antimicrobial and antifungal properties in different parts of plants have been reported.

ANTIFUNGAL TESTING

To study the antifungal activity,the fungus used were *Aspergillus spp.,Penicillium spp.and Cercospora arachidicola.*

The fungus *Aspergillus spp.* causes seedling blight or collar rot disease of groundnut (Jain & Neema 1952)[43], Black mould on onion (Shrivastava & Tiwari 1997)[44], and on garlic in Bihar (Prasad et.al.1986)[45].

Aspergillus spp. and *Penicillium spp.* causes disease to stored products. *Aspergillus spp.* and *Penicillum spp.* causes fungal contamination of red beans and peas (Bilbo et.al.2000)[46] and also causes storage fungal disease to Eucalypt seeds (Brown B.N.2000)[47].

Cercospora arachidicola causes Tikka disease of groundnut.

a) ISOLATION OF FUNGUS:

Isolation of *Aspergillus spp.*, *Penicillum spp.*and *Cercospora arachidicolal* was carried out in the laboratory. Isolation of *Aspergillus spp.*and *Penicillium spp.* were carried out by streak plate technique from contaminated jawar seeds on PDA medium.

For the isolation of *Cercospora arachidicola* the leaves of groundnut infested with Tikka disease were used. The infested leaves of groundnut were cut into small pieces, washed with

distilled water, then the material was kept in sterilised distilled water and teased with glass rod and then spread on the plate containing PDA medium and kept at room temp. ± 30°C for about 48 hrs. Thus isolated fungus were used for further testing.

b) PREPARATION OF MEDIA :

COMPOSATION OF MEDIUM :

i) Potato peeled - 20 gm

ii) Dextrose - 2 gm

iii) Agar agar - 5 gm

iv) D/W - 100 ml

The skin of potatoes were peeled off,cut into small pieces and boiled in 500ml of water till they were easily penetrated by glass rod,filtered through cheese cloth. Dextrose was added to filterate.Agar was dissolved in water,bought upto required volume 100 ml by addition of d/w,PH was

self adjusted.Then total volume was sterilised at 121^{0}C for 20 min. in autoclave.

c) STERILISATION OF GLASSWARES:

The petidishes,saline tubes, pipettes, capillary tubes, filter paper discs and other glasswares were wrapped in paper and kept in oven at 121^{0}C for 20 min.in autoclave.

d) PREPARATION OF SUSPENSION:

0.850 gm of Nacl was dissolved in 100 ml of d/w and sterilisation was carryout at 121^{0}C for about 15 min. in the autoclave. Then the spores of the *Aspergillus spp.* and *Penicillum spp.* were transferred into saline water with the help of nicrome wire loop and thus suspension was prepared.

e) PREPARATION OF PPM SOLUTION:

By dissolving the fraction of plant extract in suitable solvents, three different concentrations were prepared. The diff. concentrations prepared were 500 ppm, 250 ppm and 100 ppm. The solvents used are given in following table.

Table 1: Solvents for the preparation of solutions of the different fractions

Sr.No.	Plant	Fraction	Solvent
1	Ichinocarpus frutescens	I II	Benzene Methanol
2	Homononia riparia	I II	Pet.ether Benzene
3	Laportea interrupta	I	Benzene
4	Vernonia anthelmintica	I II	Benzene Methanol
5	Solanum surettense	I	Benzene

ANTIFUNGAL TESTING :

Antifungal testing of these plant extracts were done by paper disc method (Alice & Sivaprakasham 1996[48],Pelzar et.al. 1995[49],Mehrotra & Khan 1973[50],Atkinson & Rainsford 1946[51],Grover & Moore 1946[52]).

i) INOCULATION:

About 20 ml of sterilised PDA medium was poured in sterilised petriplates in the inoculation chamber. After solidifying,the agar plates were inoculated with 48 hrs old culture of *Aspergillus spp.* and *Penicillium spp.* with the help of angle glass rod for uniformity and even growth.In case of *Cercospora arachidicola* the spores of fungus were directly spread on the agar plates with sterilised nicrome wire loop.

ii)APPLICATION OF SAMPLE:

A good quality paper absorbent disc of 5mm diameter saturated with respective fraction was placed on surface of each streaked culture plate under aseptic conditions with the help of sterile forceps.The plates were maintained at room temperature of $\pm 30^{0}C$ for about 48 hours.The plates were observed for zone of no growth around the disc and recorded by measuring in mm.(Atkinson & Rainsford 1946)[51].

ANTIBACTERIAL TESTING

To study the antibacterial activity the bacteria *Xanthomonas axonopodis* pv *citri* were used as test organism.The *Xanthomonas axonopodis* pv *citri* cause damage to fruits of citrus by producing Brown spot (Canker) on the walls of citrus fruit,also cause Black rot of Crucifer and Leaf spot of cotton.

Xanthomonas citri is gram negative, straight, rod shaped, motile, bipolar in flagella, pale yellow in colour and aerobic in nature.

i) ISOLATION OF BACTERIA:-

For the isolation of *Xanthomonas citri* the diseased citrus fruits were taken. The section of diseased part of citrus fruit wall was taken and kept suspended in (1:1000) $HgCl_2$ solution for 3 min. Then, it was washed with d/w for 3 min. Then washed pieces of diseased part were taken into sterile saline water tubes, teased with sterilised glass rod and suspension was prepared.

ii) PREPARATION OF MEDIA :

The media used for the growth of *X.citri* was peptone agar.

Composition of Peptone agar :

Peptone	:	1 gm
Nacl	:	500 mg
D/W	:	100 ml
pH	:	6.8 to 7
Agar agar	:	3 gm

For the preparation of peptone agar 1 gm of peptone and 500 mg of NaCl was added in 100 ml D/W,and pH was adjusted to 6.8 to 7, then 3gm of agar agar was dissolved in it and whole media was sterilised at 121^{0}C for 20 min.

iii) PREPARATION OF SUSPENSION :

For the preparation of suspension about 5ml of sterile saline water was taken and yellow coloured bacterial colony was added into it with the help of sterile nicrome wire loop.

ANTIBACTERIAL TESTING:

Antibacterial testing was done by paper disc method.

INOCULATION :

About 20 ml of the sterilised medium was poured in the sterilised petriplates in the inoculation chamber. After solidifying,the suspension containing 24 hour old bacterial culture of *Xanthomonas citri* was poured into the plate and spread with the help of sterile glass rod for the uniform growth of bacteria.

v)APPLICATION OF SAMPLE :

In the centre of each petriplate the test solution was applied with the help of filter paper disc of 5mm diameter. The plates were incubated at room temp. of ± 30^{0}C for about 24 hrs.After 24 hrs.zone of inhibition were measured.

ANTIFEEDANT ACTIVITY

Many plant products are evaluated for their antifeedant activity and also reported to posses antifeedant activity against various insect pests.

The antifeedant activity was studied against two insect pests viz.

1)Red hairy catterpillar

2)Gram pod borer

1)Red hairy catterpillar

Scientific name : *Amsacta moori*

Order : Lepidoptera

Family : Aractiidae

It is a polyphagous pest and occurs on maize,jawar,sunhemp,groundnut,mung,guara,and sesamum.

Marks of Identification :

The full grown catterpillar measures about 25mm in length. Their colour varies from reddish-amber to olive green and the body is covered with numerous long hairs arising from fleshy tubercles. The moths are stoutly built and have white wings with black spots. The outer margin of fore wings,the anterior margin of thorax and the entire abdomen are scarlet red.There are black bands and dots on the abdomen.

Life cycle :

A female lays about 700-850 light yellow coloured sphirical eggs in clusters under the surface of the leaves of host plants.A single female may lay about 1,500 eggs and the eggs hatch in 2-3 days.The catterpillar grow in six stages and complete their development in 15-23 days.They pupate in soil in the earthen

cocoons.Probably more than one generation is completed in a year.

Nature of damage :

The young catterpillars prefer to eat the growing point of plants.They feed voraciously on all vegitations.

2) Gram pod borer :

Scientific Name : *Helicoverpa armigera* (Hubner)

Order : Lepidoptera

Family : Noctuidae

It is a widespread,polyphagous pest,with high mobility and fecuandity (Fitt,1989)[53].It is a serious pest of chickpea, pigeonpea, pea, mungbean, urdbean, lentil, soybean, cowpea, sorghum, okra, maize, tomato, berseem and sunflower. The pest causes high losses in pigeonpea and sorghum.(Bhatnagar et al,1982)[54].

Marks of Identification :

The moth is stoutly built and yellowish brown with dark speck and dark area near the outer margin of each fore wing.Forewings are with greyish wavy lines and black spot of varying size on the upper side and a black kidney shaped mark and a round spot on the underside.The hind wings are whitish and lighter in colour with a broad blackish band along outer margin.The full grown catterpillar is greenish with dark broken grey lines along the sides of body and measures about 3.5 cm in length.

Life cycle :

A single female may lay 741 eggs,singly in 4 days. The eggs are shining,greenish yellow and round. They hatch in 2 to 6 days.The full grown larvae measures about 35 mm and larval period lasts for 13-19 days.They pupate in soil,pupal

period lasts for 8-15 days.There may be 8 generations in a year.

Damage :

They feed on the foliage when young and on the seed in later stages.

- COLLECTION OF PEST :

The larvae of Red hairy catterpillar and Gram pod borer were collected from the village Sarnobatwadi, Dist.Kolhapur from the groundnut and gram field.They were reared in the laboratory.The Red hairy catterpillar was reared on castor leaves and Gram pod borer on cabbage leaves.

• PREPARATION OF TESTING SOLUTION :

The solutins of different concentrations (ppm) i.e. 500 ppm,250 ppm and 100 ppm were prepared from fractions separated by column chromatography, using suitable solvent.The volume of the solution prepared was about 5 ml.

• METHOD:

The antifeedant activity of the separated fractions was done by the method described by Prabal Saikia and S.Parameshwaran, 2000[55],with slight modifications.

The leaf discs of 5 cm area were punched out from the leaves of Castor, *Ricinus communis* and cabbage,then dipped into respective test solution for about a minute and air dried for 15 min.The control leaf disc were dipped in respective solvent and air dried.Then newly moulted fourth larval instar of Gram pod borer were starved for

about 24 hrs.and then released on treated leaf disc for feeding at the rate of one larvae/disc in different petridishes lined with moist filter paper. Three such replications were maintained. After 24 hrs.of feeding the excreta voided by each larvae was collected,oven dried and counted as a measure of larval feeding activity.