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EFFICACY OF SPECIFIC AQUEOUS PLANT EXTRACTS IN THE CONTROL OF SUGARCANE PATHOGEN "Curvularia lunata (Wakker Boedijn.)"

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ABSTRACT

Sugarcane has remained as an important commercial crop of agriculture and trade in India contributing substantial revenue to the exchequer by way of tax and duties. Today, it is fast transforming into the most sought after renewable energy crop, as the demand for ethanol is increasing as an alternative green fuel for the automobile. This has become far more important in the backdrop of dwindling oil reserves. Currently Brazil is diverting 50 per cent of its sugarcane for the production of ethanol and blending ethanol with petrol to the tune of 25%. India is hoping to blend ethanol at least up to 10% in the near future. It has been estimated that India will need 495 million tonnes of sugarcane by 2025 AD to meet both sugar and energy demands. Cochliobolus lunatus or Curvularia lunata is best known as the causative agent of seedling blight and seed germination failure in monocotyledon crops such as sugarcane, rice, millet, maize etc. Curvularia lunata also causes leaf spot on a wide variety of plant hosts, where each lesion contains a sporulating mass of fungi at its center. The need for method of plant protection arises because, when plants are grown close together as a crop under favourable growth conditions, far greater opportunity is provided for the spread of pests. The use of chemical fungicide carbendazim is the common practice followed by the sugarcane growers for the control of sugarcane seedling blight. The use of synthetic fungicide leads to several problems such as residue in food and feed, pathogen resistance, toxicity to

non-target organism and environmental pollution. With the increasing public awareness of environmental safety and persistent demand for eco-friendly products, we are forced to produce quality products both for export and domestic consumption. Hence, an alternative approach is the use of botanicals in management of this disease which are eco-friendly in addressing the problem. This study was undertaken to determine the inhibitory effects of extracts of plants with limited economic value against Curvularia lunata.

KEYWORDS – Chemical Fungicide, *Curvularia lunata*, Eco-friendly, Plant extract, Sugarcane

Introduction

The importance of sugar in human diet needs no introduction; it has become a part and parcel of daily life. Sugar is produced mainly from sugarcane and sugar beet and more than 75 per cent of the world sugar comes from sugarcane. Throughout ages, sugarcane remained an important commercial crop of agriculture and trade in India contributing substantial revenue to the exchequer by way of tax and duties. Today, it is fast transforming into the most sought after renewable energy crop, as the demand for ethanol is increasing as an alternative green fuel for the automobile. This has become far more important in the backdrop of dwindling oil reserves. Currently Brazil is diverting 50 per cent of its sugarcane for the production of ethanol and blending ethanol with petrol to the tune of 25%. India is hoping to blend ethanol at least up to 10% in the near future. It has been estimated that India will need 495 million tonnes of sugarcane by 2025 AD to meet both sugar and energy demands

Being as cash crop and playing major role in sugarcane economy in India this crop has affected by several pathogens *Cochliobolus lunatus* is best known as the causative agent of seedling blight and seed germination failure in monocotyledon crops such as sugarcane, rice, millet and maize. *Cochliobolus lunatus* also causes leaf spot on a wide variety of angiosperm hosts, where each lesion contains a sporulating mass of fungi at its center. The most common species infecting sugarcane is *Colletotrichum falcatum* Went, *Fusarium moniliforme* Sheldon, *Peronosclerospora sacchari, Biopolaris sacchari, C. pallescens, Ceratocystis paradoxa, Sporisorium scitamineum, Periconia atropurpurea* may also be involved in red rot disease.

Cochliobolus lunatus has a widespread distribution, though it is especially prevalent in the tropics and subtropics. Infection is caused by airborne conidia and ascospores, however, sclerotioid *C. lunatus* can also survive in the soil. The optimal temperature for *in vitro* growth

and infection ranges from 24-30 °C while death results from exposure at 59 °C for 1 minute duration, or 55 °C for 5 minute duration. Successful plant host infection requires the host surface to be wet for 13 hours. The majority of clinical cases have been reported in India, the United States, Brazil, Japan and Australia.

Curvularia is a hyphomycete (mold) fungus which is a facultative pathogen of many plant species and of the soil. Most *Curvularia* are found in the tropical region, through a few are found in temperature zones. *Curvularia* is defined by the type species *Curvularia lunata*(wakker) Boedijn. *Curvularia lunata* appears as shiny velvety-black, fluffy growth on the colony surface. *Curvularia lunata* is distinguished by septate,dematiaceous hyphae producing brown, geniculate conidiophores.

The sporoconidia are curved slightly to distinctly, transversely septate, with an expanded third cell from the pore end of the conidium. *Curvularia* can be easily distinguished from *Bipolaris* and *Drechslera* spp. Since the conidia are non-distoseptate,that is, septate from edge to edge of conidial wall. The teleomorphic state of the type species *Curvularia lunata* is *Cochliobolus*lunatus*(*fam.pleosporaceae,Ord.pleoporales,Cla.Loculoascomycetes,hy.Ascomycota).

The need for method of plant protection arises because, when plants are grown close together as a crop under favorable growth condition, far greater opportunity is provided for the spread of pests. A fungus grown on a single plant has small change of survival, but when there is a crop of many acres the condition for the multiplication and spread of the fungus may render it a factor of primary and adverse importance. Plant protection methods should ensure availability of safe and quality pesticides for sustaining crop production from the ravage of pests and diseases, streaming the quarantine measures for accelerating the introduction of new high yielding crop verities, besides eliminating the chances of entry exotic pests and for human resource development including empowerment of women in plant protection skills.

This involves the use of pesticides for control of specific disease on specific hosts; proper selection and timing of the pesticide applications are critical for control. Examples include copper and sulfur compounds. Pesticides may cause acute and delayed health effects in those who are exposed. Pesticide exposure can cause a variety of adverse health effects. These effects can range from simple irritation of the skin and eyes to more severe effects such as

affecting the nervous system, mimicking hormones causing reproductive problems, and also causing cancer. A 2007 systematic review found that "most studies on non-Hodgkin lymphoma and leukemia showed positive associations with pesticide exposure" and thus concluded that extensive use of pesticides should be decreased. Strong evidence also exists for other negative outcomes from pesticide exposure incuding neurological, birth defects, fetal death and neuro developmental disorder.

The use of chemical fungicide carbendazim is the common practice followed by the sugarcane growers for the control of sugarcane seedling blight. Practicing set treatment with systemic fungicides alone is not sufficient to protect such a long duration crop. The use of synthetic fungicide leads to several problems such as residue in food and feed, pathogen resistance, toxicity to non target organism and environmental pollution (Angelo et al. 2012). In addition to these, elimination of soil born inoculum through chemicals is difficult and costly. Development of resistant varieties through breeding methods is a long term endeavour. Therefore, with the increasing public awareness of environmental safety and persistent demand for ecofriendly products, we are forced to produce quality products both for export and domestic consumption. Hence, an alternative approach is the use of botanicals in management of this disease which are eco friendly in addressing the problem.

It has been suggested that combining plant extracts with nonchemical postharvest treatments, such as heat treatment, might control postharvest red rot diseases effectively .However, the antifungal substances that are present in the plant extracts may be unstable at higher temperatures .Accordingly, there is a need for investigating a heat stable plant extract that could be integrated with hot water treatments. This study was undertaken to determine the inhibitory effects of extracts of plants with limited economic value against *C. lunata.*

Material And Methods

I. Collection of Leaves from Plants with Common Uses and Therapeutic Uses

Fresh leaves of 21 plant species were collected from Western Ghats, Theni District. The plants included common uses plants like *Moringa pterigosperma, Mangifera indica, Citrus sinensis, Carica papaya linn, Eclipta albahassll, Ficus bengalensis, Pongamia glabra vent, Hibiscus rosasinensis linn,* therapeutic used plants like *Sesbania grandiflora, Cynodon dactylon, Syzygium cumini, Andrographis paniculata, Chamaecostus cuspidatus, Aerva lanata,*

Aealypha indica, Vitrex negunda linn, Ocimum basilicum, Xanthium strumarium, Leucas asperaspreng, Indigofera tinctoria linn, Aegle marmelos.

II.Preparation of Crude Leaf Extracts

- The 21 plant samples were washed in fresh water to remove the adhering dust and then dried

  under shade. The collected 21 plant leaves were left at room temperature for two weeks to dry.
- Leaves were chopped into small pieces and then ground into powder. The finely ground material (50 gm) was extracted with 500ml of aqueous in conical flask in shaking condition.
- The extract was decanted into new conical flask. The process was repeated 3 times with the same material but using fresh water.
- Soxhlet extractor was used successively with water. The successive extracts were evaporated to dryness and the lyophilized residue was used for further analysis.
- The extracted residues were weighed and re-dissolved in sterile distilled water to yield 1µg/ml solutions ready for further analysis.

III.Phytochemical Analysis of Aqueous Leaves Extract

The successive extracts (aqueous) of 21 plant species were subjected to preliminary phytochemical screening for the identification of its active constituents in a qualitative way by the method of Harborne (1998) and Kokate(2001).

IV. Collection Of Diseased Sugarcane Leaves

Disease infected Sugarcane (blight disease) leaves were collected from sugarcane fields to isolate the causal organism from the leaf spots. The study areas includes paddy fields in and around in Theni District like Allinagaram, Bommayagoundan patti,etc. Leaves were collected from sugarcane field which was shown during the month of December.

V. Isolation of Sugarcane Pathogen

Materials Required:

1. Infected Sugarcane Leaves
2. Sterile Blades
3. Sterile Forceps
4. Sterile Filter Paper

5. Water Agar (2%)
6. 70% Ethanol _ 70ml of ethanol is taken and the volume made up to 100ml using 30ml of distilled water.
7. 0.1% Mercuric chloride (Hg_2Cl_2) _ 0.1g of Hg_2Cl_2 dissolved in 100ml of distilled water.
8. Potato Dextrose Agar
9. Sterile Petridishes:

The infected leaves were cut into small pieces using sterile blade. The spots were retained along with the small cut pieces. They were put in a beaker and surface sterilized as per the following procedure:

Using sterile forceps, the leaf pieces were taken out and dried by tapping them onto sterile filter paper. The leaf pieces were transferred into 2% water agar with the abaxial side of the leaf spot touching the surface of the medium. The plates were incubated at room temperature. After 48 hours, mycelium from some leaf spots and the leaf bits were transferred into PDA plates. The individual fungal colonies were sub cultured onto PDA slants for further identification.

VI.Identification Of Fungal Pathogen- Slide Culture Method

- The PDA plates were taken and a small disc of 5mm dia was made using sterile cork borer.
- A moist chamber was made by placing sterile cotton on the lower cover of an empty petri dish.
- One disc was placed on top of the microscopic slide using an inoculation loop.
- Fungal culture was inoculated on the disc.
- A cover slip was pressed gently on the top of the disc with culture.
- After an incubation of 48-72 hrs, the slides were stained with Lacto phenol cotton blue and observed under a compound microscope.
- The size, shape and number of conidia were studied and recorded.

VII.Antifungal Assay: Disc Diffusion Method

Materials Required

1) PDA Medium
2) *Curvularia* culture
3) Plant extracts
4) Sterile L-Rod
5) Whatmann 1 filter paper
6) Sterile distilled water
7) Petridishes

- 5ml of culture suspension of Curvularia with a concentration of $1*10^5$ conidia ml^{-1} was added to 250ml PDA medium. Allowed to solidify.
- Sterile filter paper discs (5mm in diameter) were impregnated with 10 µl plant extract (at a concentration of 10 µg/µl) , sterile water and commercial fungicide (carbendazim).
- The 23 plates were incubated in 48 to 72 hours at 28ºC
- Inhibitory zone was measured on a 0 – 4 scale.
- Triplicates were maintained for the above treatment.

VIII.Thermal Inactivation Of Plant Extracts:

Plant extracts showing potential antifungal activity in the preliminary evaluation were further tested for thermal stability. About 1 ml of aqueous extracts in glass tubes were exposed to 60°C in a water bath for 10 min and cooled to room temperature. Afterwards, the paper disc method was employed to assess their effect on antifungal activity against *C. lunata* as described.

IX.Spore Germination Assay:

- The plant extracts which showed specific inhibitory effect towards the sugarcane pathogen, *Curvularia lunata* were taken for further analysis.
- In this assay, about 0.5 ml of aqueous plant extract was pipetted into sterile test tubes and the solvent was allowed to evaporate at room temperature.
- About 4.5 ml volume of potato dextrose broth was pipetted into the test tubes and mixed with extracts.
- At the same time an aliquote (100 µl) of spore suspension (adjusted to 10^5 conidia ml-1) was added into each tube.
- After 24 hrs of incubation at 28°C on a rotary shaker, a drop of the mixture from each tube were placed in a microscope slide and slides were fixed in lactophenol cotton blue and observed microscopically for spore germination.
- The same volume of sterile water was added in place of plant extracts in the control samples and carbendazim at the concentration of 10 mg/ml served as a positive control.
- A conidium was considered as germinated if the length of the germ tube was at least half the length of the conidium.
- The number of germinated conidia was counted out of 100 randomly selected conidia in three replicate slides.
- Percentage spore germination was calculated according to the following formula:

Spore Germination (%) = (Germinated Spores (No.) / (Total Spores (No.)) ×100

X. Comparision Of Antifungal Acitvity With Two Benificial Fungus

The process was done to compare the inhibitory effect of plant extract on two beneficial fungus *Aspergillus niger* and *Pencillum notatum.*

- 5ml of culture suspension of *Aspergillus niger* and *Pencillum notatum* with a concentration of $1*10^5$ conidia ml^{-1} was added to 250ml PDA medium. Allowed to solidify.
- Sterile filter paper discs (5mm in diameter) were impregnated with 10 µl plant extract (at a concentration of 10 µg/µl) , sterile water and commercial fungicide (carbendazim).
- The 21 plates were incubated in 48 to 72 hours at 28ºC .

XI.Quantitative Analysis Of Crude Extract

Phenols:

The solvent extracts were used for the determination of the total phenolics by spectrophotometrically according to the Folin-Ciocalteau colorimetric method (Singleton & Rossi, 1965). Each extract (200 µL) was introduced into screw cap test tubes and 1.0 mL of Folin-Ciocalteau reagent (1:1 with water) and 1.0 mL of sodium carbonate (7.5%) were added. The tubes were vortexed incubated for 2 h and the absorbance was read at 726 nm using a spectrophotometer (Beckman, USA) The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.

XII.UV Spectrum Analysis Of Plant Extracts

UV–Vis spectra were recorded using a UV–Vis spectrophotometer UV 1800 (Shimadzu, Japan) between 200 and 800 nm. Methanol was used as blank. The extracts were examined under visible and UV light for proximate analysis. For UV-VIS spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent.

XIII.FT-IR Spectrum Analysis Of Plant Extracts

IR spectra for the aqueous crude extract were recorded on a Perkin–Elmer 1600 series FTIR spectrometer using KBr pellets. which was used to detect the characteristic peaks and their functional groups.

XIV.GC-MS Analysis Of Plant Extracts

Gas Chromatography: An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35^{o}C, hold for 2 minutes, then ramp at 20^{o}C per minute to 300^{o}C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).

Mass Spectrometry

A JEOL GCmate II benchtop double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000^{1} software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Mass spectrometry library search

Identification of the components of the purified compound was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software.

Identification Of Phytocompounds

Interpretation on Mass-Spectrum GC-MS was conducted using the dadabase of National institute Standard and Technology (NIST)having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the Components of the test materials were ascertained.

Results

I. Collection Of Leaves From Plants With Common Uses And Therapeutic Uses:

II. Dried leaves of about 21 different plant species with common and therapeutic uses were obtained. (Plate- 1)

II.Preparation of Crude Leaves Extract

Aqueous crude leaf extract was prepared from the dried leaves by subjecting the samples to Soxhalator extraction (Plate-2).Dry weight was measured (Table-1).

Plate-2

Table-1 a

Crude Extract

Common Plant List

s.no	Botanical Name	Dish with extract(gm)	Extract weight(gm)
1	*Moringa pterigosperma*	*4.875*	*1.128*
2	*Mangifera indica*	*4.971*	*1.224*
3	*Citrus sinensis*	*4.014*	*1.267*
4	*Carica papaya linn*	*5.086*	*1.339*
5	*Eclipta albahassll*	*4.899*	*1.152*
6	*Ficus bengalensis*	*4.806*	*1.059*
7	*Pongamia glabra vent*	*4.844*	*1.097*
8	*Hibiscus rosasinensis linn*	*4.840*	*1.093*

Table 1 b

Crude Extract Weight

Herbal Plant List

Sl.no	BOTANICAL NAME	Dish with extract(gm)	Extract weight(gm)
1	*Sesbania grandiflora*	*4.894*	*1.147*
2	*Cynodon dactylon*	*4.816*	*1.069*
3	*Syzygium cumini*	*4.915*	*1.168*
4	*Andrographis paniculata*	*5.068*	*1.321*
5	*Chamaecostus cuspidatus*	*4.897*	*1.15*
6	*Aerva lanata*	*5.636*	*1.889*
7	*Aealypha indica*	*4.880*	*1.133*
8	*Vitrex negunda linn*	*4.843*	*1.096*
9	*Ocimum basilicum*	*4.948*	*1.201*
10	*Xanthium strumarium*	*5.638*	*1.891*
11	*Leucas asperaspreng*	*5.448*	*1.701*
12	*Indigofera tinctoria linn*	*4.870*	*1.123*
13	*Aegle marmelos*	*5.075*	*1.328*

III.Phytochemical Analysis of Aqueous Leaves Extracts

In the present study, the preliminary phytochemical analysis of 21plant leaf extracts (aqueous) confirms the presence of Saponins ,Proteins ,Quinones , Flavonoids ,Reducing sugar , Phenolic compound ,Alkaloids ,Tannins ,Glycosides and Terpenoids (Table-3).

Table-2 a Phytochemical Analysis of Common Plants

S. no	BOTANICAL NAME	Saponins	Proteins	Quinones	Flavonoids	Reducing sugar	Phenolic compound	Alkaloids	Tanins	Glycosides	Terphonoids
1	*Moringa pterigosperma*	+	–	+	+	+	+	+	+	+	+
2	*Mangifera indica*	+	+	+	–	–	+	–	+	–	+
3	*Citrus sinensis*	+	+	+	+	+	+	+	+	+	+
4	*Carica papaya linn*	+	–	+	+	+	+	+	+	–	–
5	*Eclipta albahassll*	+	+	+	–	–	+	+	–	–	+
6	*Ficus bengalensis*	+	–	+	+	–	+	+	+	+	+
7	*Pongamia glabra vent*	+	+	+	–	–	+	+	–	–	–
8	*Hibiscus rosasinensis linn*	+	+	+	+	–	+	–	+	+	+

Table-2 b Phytochemical Analysis of Herbal Plants

S.NO	BOTANICAL NAME	Saponins	Proteins	Quinones	Flavonoids	Reducing sugar	Phenolic compound	Alkaloids	Tanins	Glycosides	Terphonoids
1	*Sesbania grandiflora*	+	–	+	+	–	+	+	+	+	+
2	*Cynodon dactylon*	+	+	–	+	+	+	–	+	+	–
3	*Syzygium cumini*	+	+	+	+	–	+	+	+	+	+
4	*Andrographis paniculata*	+	+	+	+	+	+	–	–	–	–
5	*Chamaecostus cuspidatus*	+	–	–	+	–	–	–	–	–	–
6	*Aerva lanata*	+	+	+	+	+	+	+	+	+	+
7	*Aealypha indica*	+	–	+	–	–	+	–	+	–	–
8	*Vitrex negunda linn*	+	–	+	–	–	+	–	+	+	–
9	*Ocimum basilicum*	+	+	+	+	-	+	+	–	+	–
10	*Xanthium strumarium*	+	+	+	+	+	+	–	–	+	–
11	*Leucas asperaspreng*	+	–	+	+	–	+	–	+	+	–
12	*Indigofera tinctoria linn*	+	–	+	–	–	+	+	–	+	+
13	*Aegle marmelos*	+	+	+	+	+	+	–	+	+	+

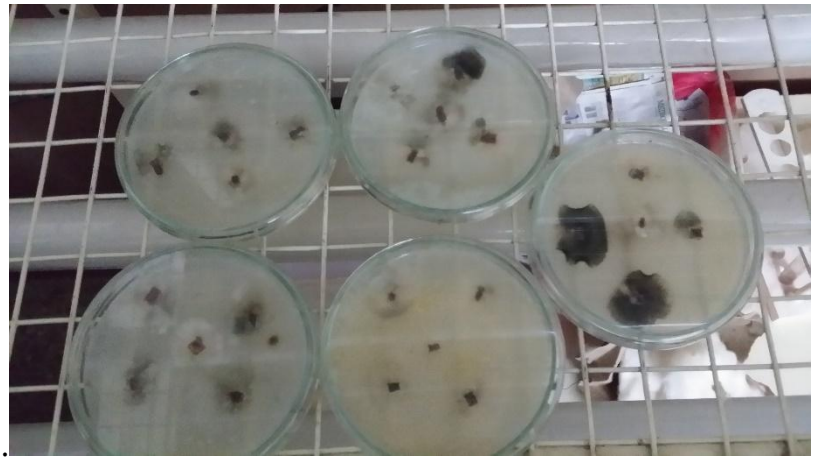
IV.Collection of Diseased Sugarcane Leaves

The infected sugarcane leaves with elongated red spots which had coalesced together was collected. These spots had a blight like appearance. The leaves were exhibiting a bit of necrosis too. Leaf bits with the characteristic spots were used for isolation of the pathogen (Plate-3).

Plate-3

V.Isolation Of Sugarcane Pathogen

The surface sterilized leaf bits were inoculated onto water agar plates. These plates were kept for incubation at room temperature. Mycelial growth was observed around the spots in some plates after 3-4 days. These leaf bits were transferred onto Potato Dextrose Agar plates and kept for incubation again at room temperature. Some of the spots showed abundant colony formation after two days and these were marked and maintained in PDA slants for identification. (Plate-4) **Plate-4**

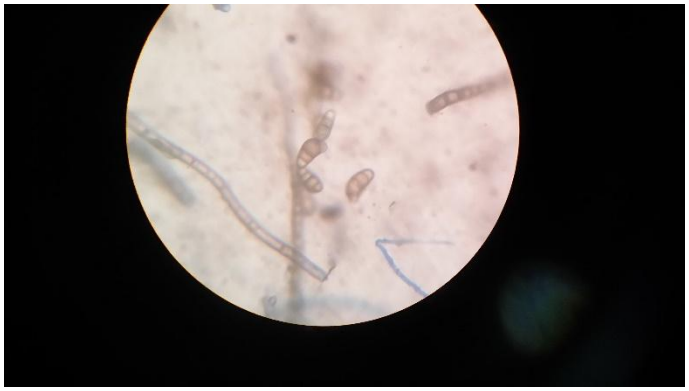


VI.Identification Of Fungal Pathogen- Slide Culture Method

The fungal mycelium was stained with Lactophenol cotton blue. It was observed under 40X using a Light Microscope. Detailed notes were made on A) the nature of conidiophores, B)the

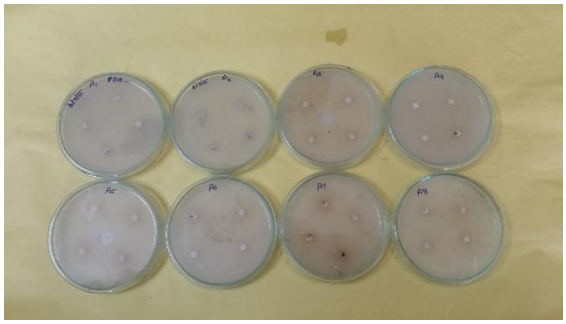
arrangement of conidia on conidiophores and C) the shape, size and color of the conidia. The conidiophores were erect, stiff and olive to black and had an acropleurogenous arrangement of conidia on it. Most of the conidia were curved or row-boatshaped. But straight shaped conidia were also present. They were mostly triseptate and rarely tetraseptate. They were oilvaceous black to dark brown in color. The third cell from the base was largest and the end cells were subhyaline or pale brown in colour (PLATE-5). When the conidia happened to be straight, the apical cell appeared rounded. By comparing with the literature given on *Curvulaia lunata* , the type species identification , the isolation fungi was confirmed to be *Curvulaia lunata*, the type species defined by (Wakker) Boedijn, (1933).

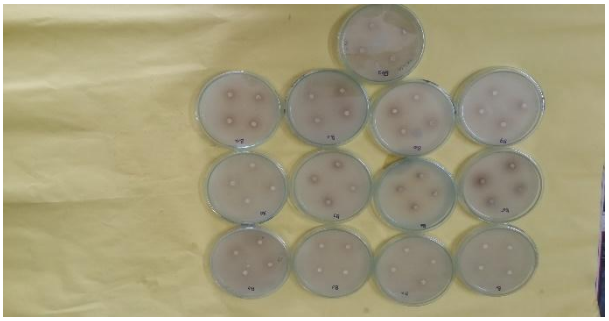
Plate-5

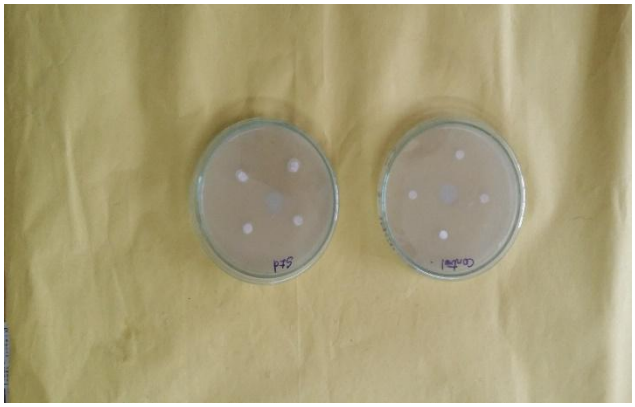


VII.Antifungal Assay: Disc Diffusion Method

The antifungal activity of 21 aqueous plants leaf extracts tested against *Curvulaia lunata* are shown in Table 4. The antifungal activity of these plant extracts was noted on *Aspergillus niger* and *Penicillum notatum* also (Plate-6). A minimum inhibitory concentration range was also plotted for the plant extracts which showed specific inhibitory activity towards *Curvularia lunata*. (Table-4)







VIII.Spore Germination Assay

Spores were counted under 40X objective of compound microscope. The spore germination percentage was seen from 1.5% to 2% in the samples treated with plant extracts which showed inhibitory action towards *Curvularia lunata*(table-5). This showed proof that there was more than 95% of fungal inhibition using the plant extracts.

Common Plants

PLANT SPECIES	ZONE OF INHIBITION(cm)			
	1	2	3	Average
A1	-	-	-	-
A2	-	-	-	-
A3	-	-	-	-
A4	-	-	-	-
A5	-	-	-	-
A6	2.8	2.7	2.8	2.8

A7	**4.0**	**4.1**	**4.0**	**4.0**
A8	-	-	-	-
STANDARD	4.8	4.8	4.7	4.8
CONTROL	-	-	-	-

Herbal Plants

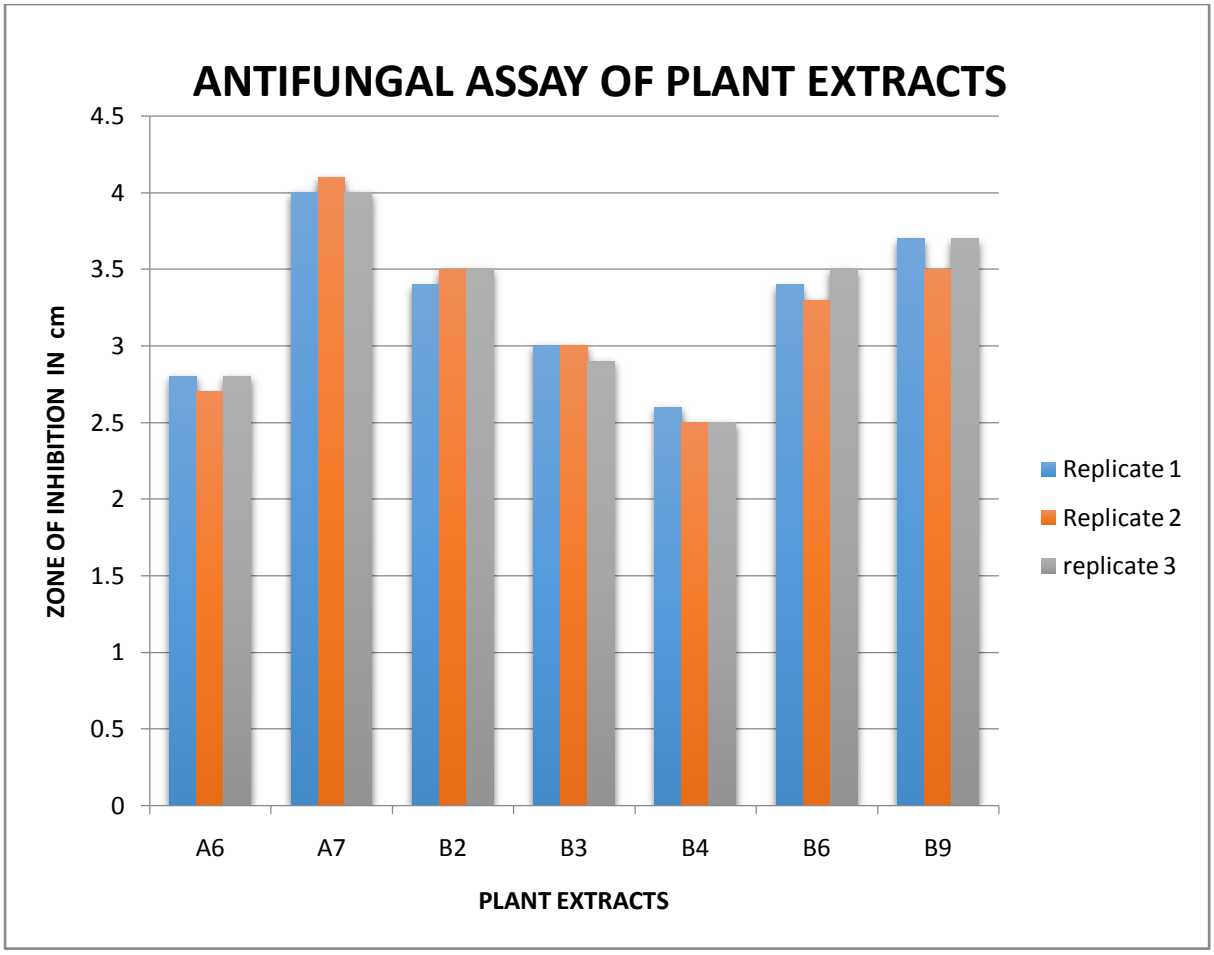
PLANT SPECIES	**ZONE OF INHIBITION(cm)**			
	1	**2**	**3**	**Average**
B1	-	-	-	
B2	3.4	3.5	3.5	3.5
B3	3.0	3.0	2.9	3.0
B4	2.6	2.5	2.5	2.5
B5	-	-	-	
B6	3.4	3.3	3.5	3.5
B7	-	-	-	
B8	-	-	-	
B9	3.7	3.5	3.7	3.7
B10	-	-	-	-

B11	-	-	-	-
B12	-	-	-	-
B13	-	-	-	-

Effect of aqueous extracts of selective plant species on conidial germination of *Curvularia lunata(*wakker)

PLANT SPECIES	**CONIDIAL GERMINATION (%)**
A6- *Ficus bengalensis*	**1.5**
A7- *Pongamia glabra vent*	**0.5**
B2- *Cynodon dactylon*	**0.3**
B3- *Syzygium cumini*	**0.9**
B4- *Andrographis paniculata*	**1.0**
B6- *Aerva lanata*	**0.5**
B9- *Ocimum basilicum*	**0.6**
Carbendazamin	**0.8**
Control	**98.9**

Percentage of germinated spores 24 h after incubation (mean of three replication)



IX.Thermal Inactivation Of Plant Extracts

The antifungal activity of the plant extracts observed after thermal inactivation was in concordance with the antifungal activity of the plant extract prior to thermal inactivation. (Table-6).

Thermal inactivation of *in vitro* activity of seven plant extracts against *Curvularia lunata* four days after incubation.

TEMPERATURE (OC)	**PLANT SPECIES**	**ZONE OF INHIBITION (cm)a**
60^0C	**A6-** *Ficus bengalensis*	**2.9**
	A7- *Pongamia glabra vent*	**3.9**
	B2- *Cynodon dactylon*	**3.3**
	B3- *Syzygium cumini*	**3.5**

	B4- *Andrographis paniculata*	**2.7**
	B6- *Aerva lanata*	**3.7**
	B9- *Ocimum basilicum*	**3.6**
	Carbendazamin	**3.9**
	Control	**-**

Values are means of three replications.

X.Quantitative Analysis Of Selective Plant Extract

Phenols

The total phenolic content was measured using spectrophotometrically according to the Folin-Ciocalteau colorimetric method. The highest total phenolic content was observed in *Cynodon dactylon* (225mg GAE/g) followed by *Syzygium cumini* (82.5mg GAE/g) and *Ficus bengalensis*(21mg GAE/g),respectively.

TOTAL PHENOLS IN SPECIFIC PLANT EXTRACTS

Sample	**Total phenol content***
A6- *Ficus bengalensis*	21 mg
B3- *Syzygium cumini*	225 mg
B4- *Andrographis paniculata*	82.5 mg

*****total phenol content was expressed as mg gallic acid equivalent/gram dry extract

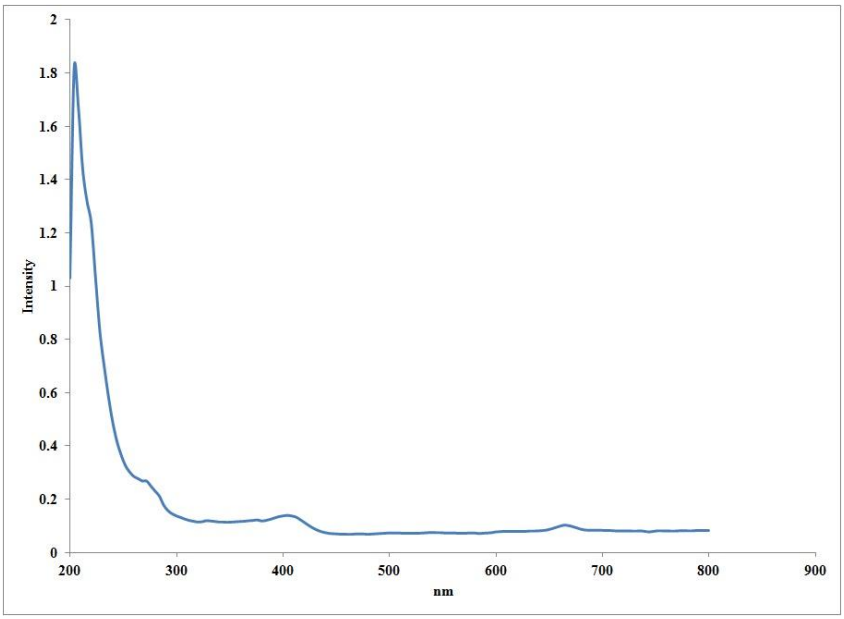
XI.UV Spectrum of Plant Extract

The UV-VIS profile of plant extract was taken at the 200 to 800nm wavelength due to the sharpness of the peaks and proper baseline. In Ficus *bengalensis*,a peak absorption value of 1.85 was observed at 250nm. And in *Cynodon dactylon*,two peaks were observed with an

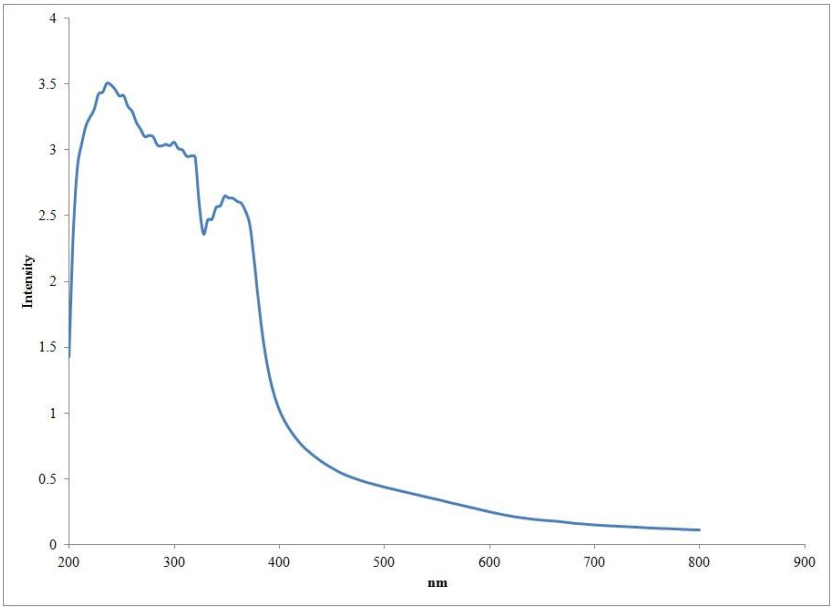
absorption value of 3.5 at 240 nm and an absorption value of 2.7 at 350nm. In *Syzygium cumini* ,three peaks were observed with an absorption value of 0.138 at 205 nm ,an absorption value of 0.102 at 300 nm and an absorption value of 0.102 at 340nm.(Figure-)

A1- *Ficus bengalensis* B2- *Cynodon dactylon* B3-*Syzygium cumini*

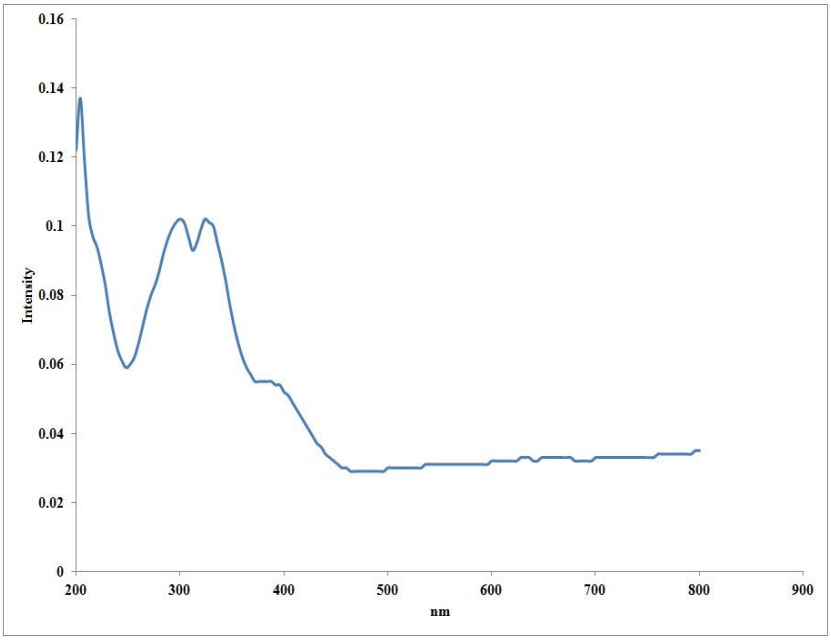
A1



B2

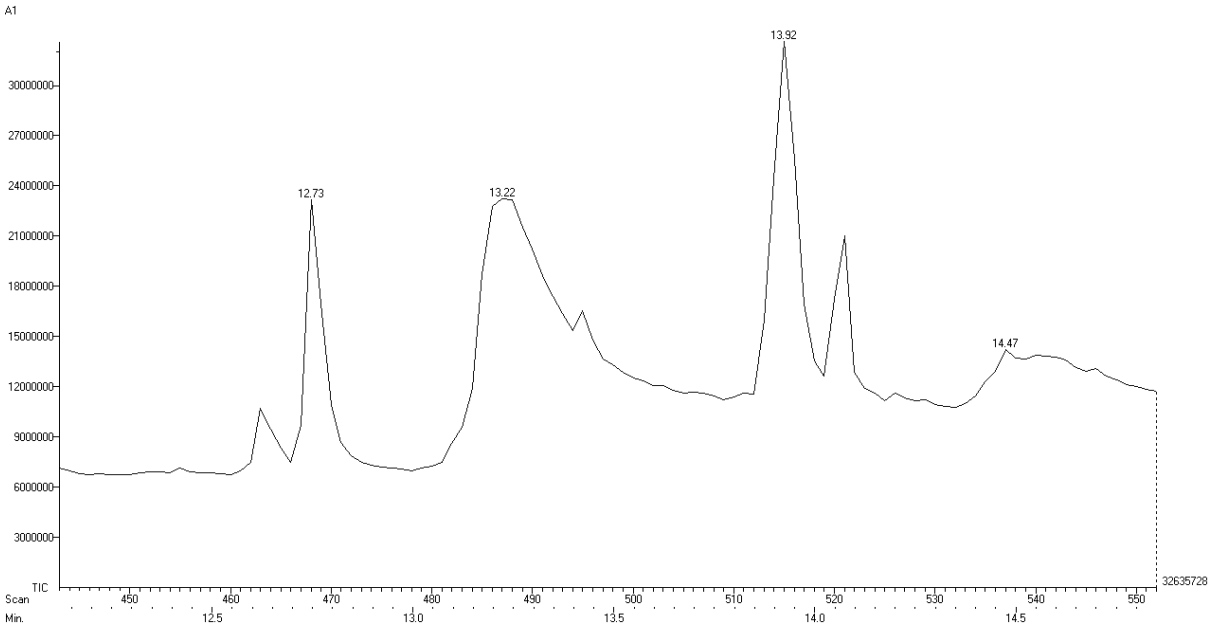


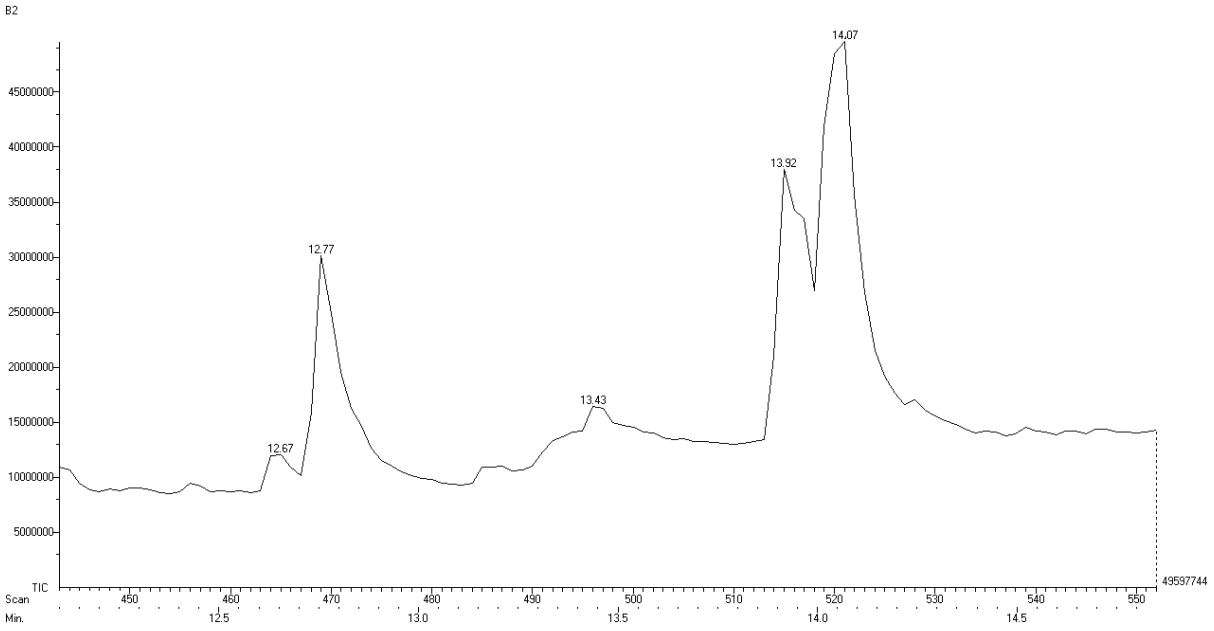
B3

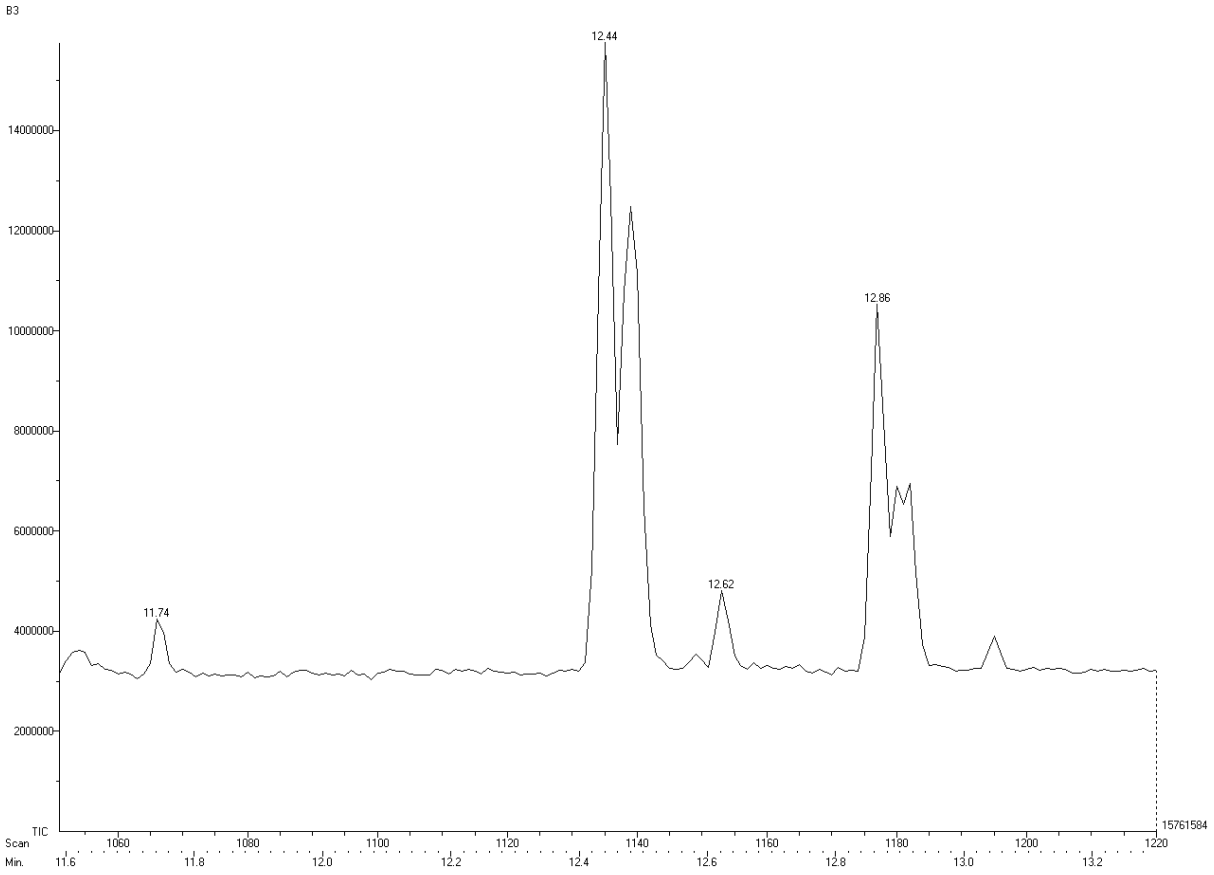


XII.FTIR Spectrum of Plant Extract

The FTIR spectrum was used to identify the functional groups of the active components present in plant based on the peaks values in the region of IR radiation. When the plant extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of alcohol, phenol, alkanes, 1^0, 2^0 amines, aldehydes, aromatic compound, secondary alcohol, aromatic amines and halogen compound. (Table-8)



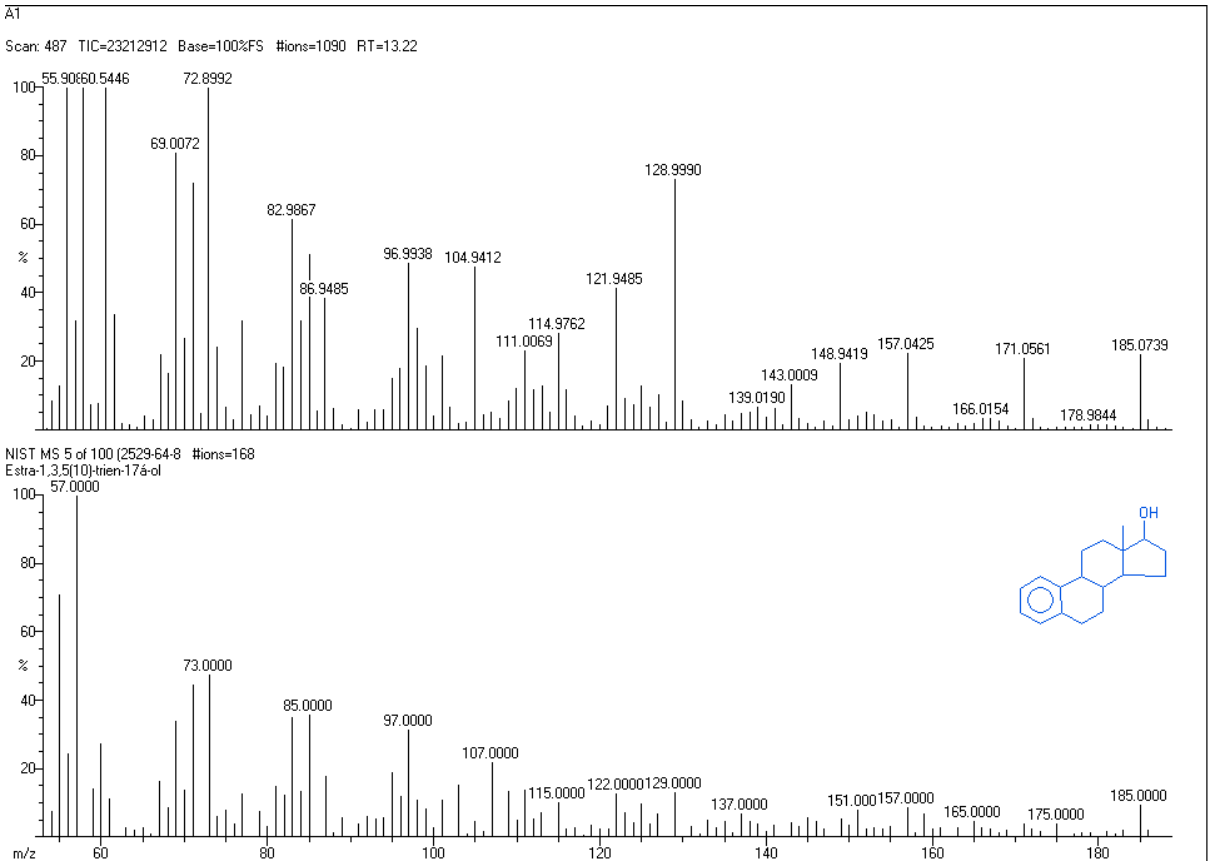


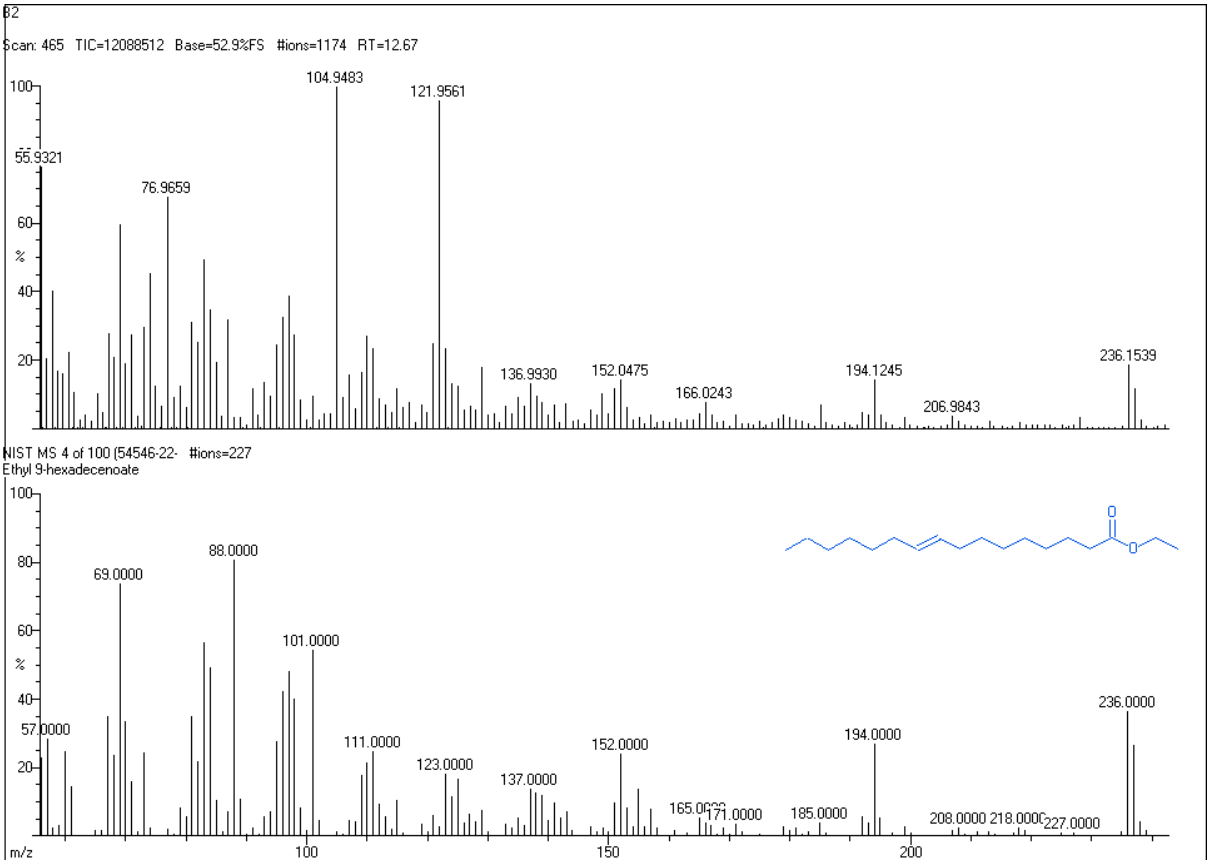


XIII.GC-MS Analysis of Plant Extract

The bioactive compounds of 3 aqueous plant extracts have been evaluated using GC-MS. The chemical compositions of the whole plant aqueous plant extract of 3 plants were investigated using Perkin-Elmer Gas Chromatography-Mass Spectrometry, while the massspectra of the

compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library(Table-9).




82
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55.9321
76.9659
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NIST MS 4 of 100 (54546-22- #ions=227
Ethyl 9-hexadecenoate
88.0000
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165.0000
171.0000
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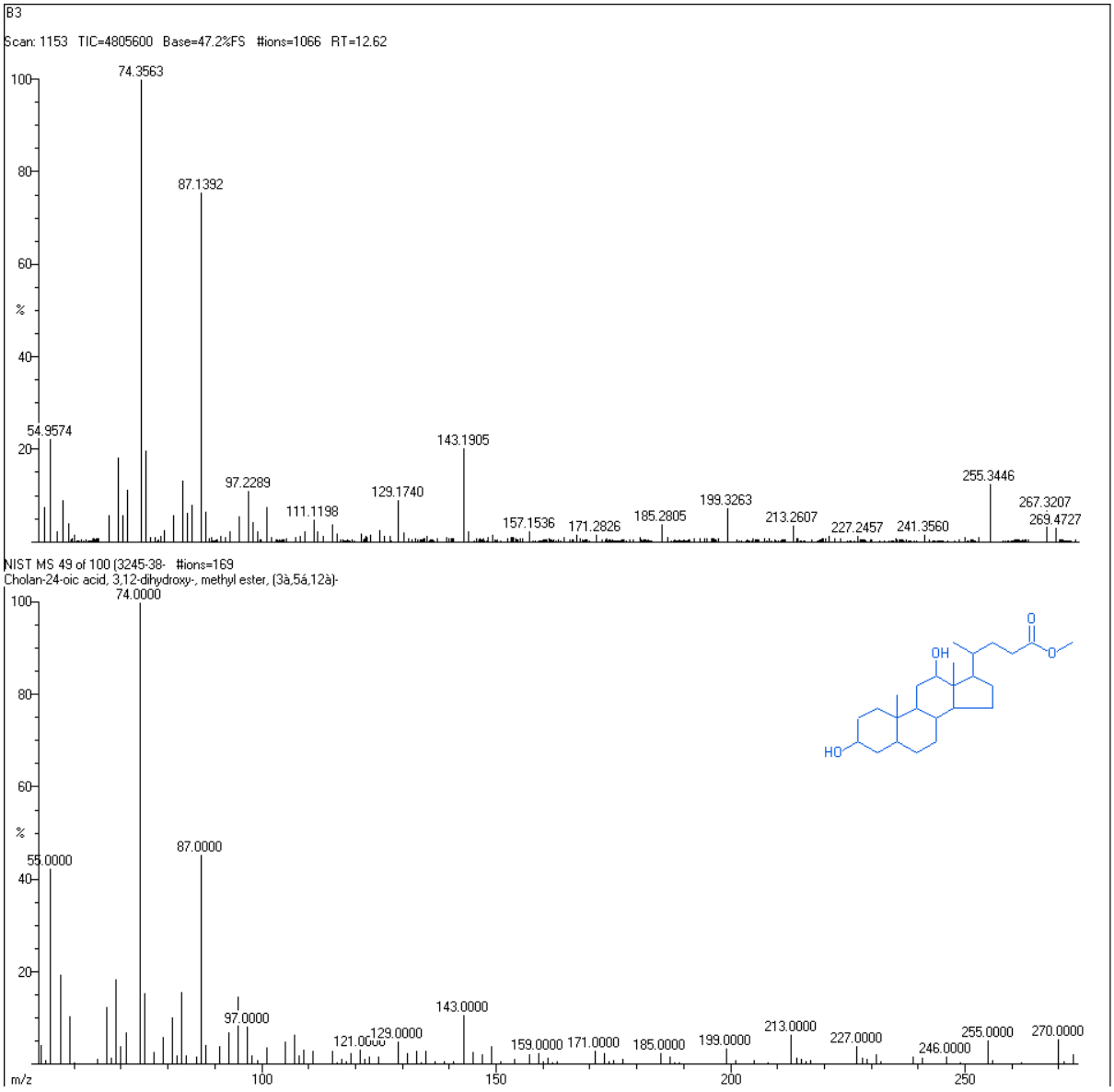
208.0000
218.0000
227.0000
236.0000
m/z



Conclusion

Around 21 crude leaf extracts from plants with common as well as therapeutic uses were obtained. A qualitative phytochemical analysis was done on these crude leaf extracts to screen for the active constituents present in them. It showed the presence of various secondary metabolites such as Saponins , Proteins, Quinones ,Flavonoids, Reducing sugars, Phenolic compounds, Alkaloids, Tannins, Glycosides, Terpenoids. A preliminary study of selective plant extracts and their control on the sugarcane pathogen *Curvularia lunata* has been carried out. An antifungal assay by disc diffusion method and a spore germination assay was done. Around seven plant extracts of *Ficus bengalensis, Pongamia glabra vent, Cynodon dactylon, Syzygium cumini, Andrographis paniculata, Aerva lanata, Ocimum basilicum.* A zone of inhibition in the range of showed upto 2.5 to 4.0 cm range and spore germination range of 1.5 to 2% which indication more than 95% of fungal inhibition ,which was greater

than when compared to commercial fungicide. A minimum inhibitory fungicide. A concentration assay was also done and the IC_{50} range was found to be between 2µg /µl to 10µg/µl. a thermal inactivation experiment of the plant extracts was carried out to determine the stability of the antifungal activity of the plant extracts against the sugarcane pathogen *Curvularia lunata*. The results obtained were in concordance with the value prior to thermal inactivation of the plant extracts. Some of the plant extracts which showed an inhibitory action on *Curvularia lunata* were categorized as 'risky' since they showed inhibitory activity with *Aspergillus niger*, *Penicillum notatum.* So they had a broad spectrum action. Only three plants extracts of *Ficus bengalensis, Cynodon dactylon, Syzygium cumini* were specific towards *Curvularia lunata.* These three plants extract were taken for further analysis because of their narrow spectrum action. In UV vis spectroscopy relevant peaks were obtained at a range of 205nm to 350nm. This indicated the presence of bioactive compound. These samples were subjected to FT-IR analysis. FTIR analysis confirmed the presence of alcohol, phenol, alkanes, 1^0,2^0 amines,aldehydes, aromatic compound, secondary alcohol, aromatic amines and halogen compound. The results pertaining to GCMS Analysis led to the identification of a number of compounds from the GC fractions of the aqueous extracts of the three plant species *Ficus bengalensis, Cynodon dactylon, Syzygium cumini.* These compounds were identified through mass spectroscopy attached with GC.The phytochemicals identified have been shown to possess antipyretic,anticancer, germicidal and antimicrobial activities.

The present study was done to analyse the antifungal activity of selective plant extracts against the sugarcane pathogen, *Curvularia lunata*(Wakker) Boedijin.These samples were further subjected to spectroscopic and chromatographic techniques to identify the phytoconstituents present in them. Many of the bioactive compounds identified were known to have antipyretic, anticancerous, germicidal and antimicrobial properties according to previous reports. These plant extracts can be used for field application instead of commercially available chemical fungicides. Many phytopreparations of different concentrations with fungicidal properties can be prepared from these plant extracts.Phytopreparations are ecologically safe, do not leave negative effect on treated plants, their usage pays off and they are suitable for prophylactic treatment.

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TABLES AND FIGURES

Common Plant Leaves List (Table-1)

NAME	COMMON NAME	BOTANICAL NAME	ENGLISH NAME
A1	Muruggai ilai	*Moringa pterigosperma*	Drumsticks leaves
A2	Ma ilai	*Mangifera indica*	Mango leaves
A3	Orange ilai	*Citrus sinensis*	Orange leaves
A4	Papali ilai	*Carica papaya linn*	Papaya leaves
A5	Karisilakanni	*Eclipta albahassll*	False daisy
A6	Alam ilai	*Ficus bengalensis*	Banyan tree leaves
A7	Pungai ilai	*Pongamia glabra vent*	Indian beach leaves
A8	Separuthi	*Hibiscus rosasinensis linn*	Shoe flower

Herbal Plant Leaves List (Table-1)

NAME SUBSTITUTE	COMMON NAME	BOTANICAL NAME	ENGLISH NAME
B1	Agathi ilai	*Sesbania grandiflora*	August tree leaves
B2	Arugampul ilai	*Cynodon dactylon*	Bermuda grass leaves
B3	Naaval ilai	*Syzygium cumini*	Blackberry leaves
B4	Nilavebbu ilai	*Andrographis paniculata*	Green chirayta leaves
B5	Insulin ilai	*Chamaecostus cuspidatus*	Fiery costus leaves
B6	Kanthulai ilai	*Aerva lanata*	Aerva lanta leaves
B7	Kuppai meni ilai	*Aealypha indica*	Cat's straggle leaves
B8	Karu Nochi ilai	*Vitrex negunda linn*	Chaste tree leaves
B9	Thulasi	*Ocimum basilicum*	Sweet basil leaves
B10	Marul umathan	*Xanthium strumarium*	Marul umathan leaves
B11	Thumbai	*Leucas asperaspreng*	Leucas leaves
B12	Auri	*Indigofera tinctoria linn*	Aviri leaves
B13	Vilvam	*Aegle marmelos*	Beal tree leaves