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TLC AND GCMS BASED IDENTIFICATION OF ANTIFUNGAL COMPOUNDS TO CONTROL BROWN MUSCARDINE, ASPERGILLUS NIGER INFECTING CHAWKI WORMS OF MULBERRY SILKWORM, BOMBYX MORI L

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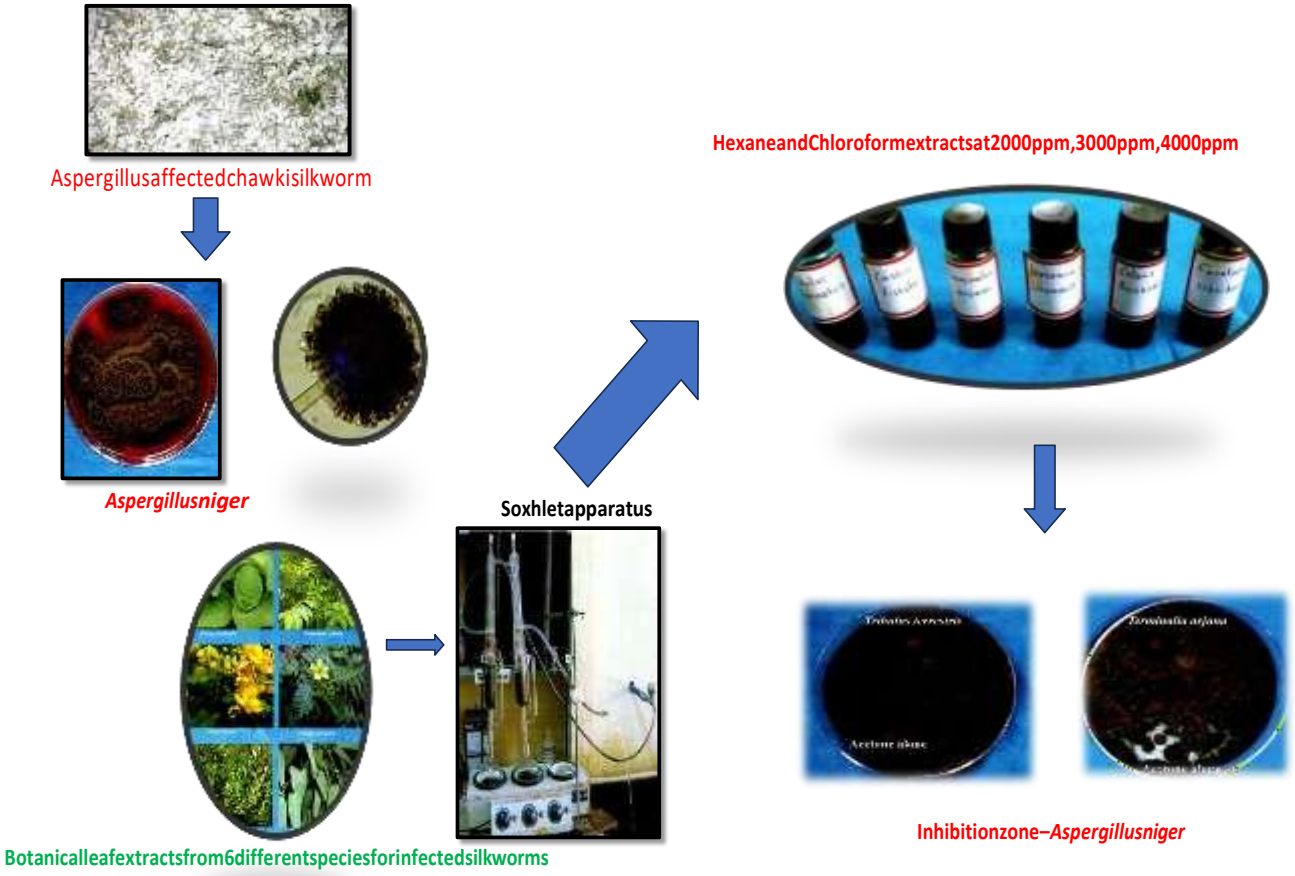
ABSTRACT

Aspergillosis is serious a fungal disease in young age worms or chawki worms of mulberry silkworm, Bombyx mori. It inflicts a loss upto 30 per cent in various districts of Tamil Nadu. At present there is more number of Chawki Rearing Centre in Tamil Nadu, though a good sign of improvement but this disease may cause havoc in years to come. Hence the present problem was taken up during 2023-2025 to study the etiology and management of Aspergillosis. In vitro studies were conducted with chloroform and hexane leaf extracts of botanicals against A. niger. Hexane extract of the T. arjuna against A. niger gave maximum inhibition zone of 19.8, 21.0 and 22.8mm at 2000, 3000 and 4000ppm respectively. This was followed by C. fistula (19.0mm, 20.1mm and 21.0mm) and T. terrestris (15.1mm, 16.2mm, 17.8mm). The chloroform extract of the T. arjuna against A. niger were found to have maximum inhibition zone of 20.8, 21.9 and 25mm at 2000, 3000 and 4000ppm concentrations respectively. This was followed by C. fistula (17.6, 20.1and 21.9 mm) and T. terrestris (16.1, 17.9 and 20.1mm). Based on the in vitro studies on evaluating the efficacy of botanicals against A. niger chloroform leaf extracts of T. arjuna and T. terrestris showed maximum antifungal activity when compared to other plant leaf extracts. Studies using Thin Layer Chromatography (TLC) plate revealed good resolutions with [Rf values (relation to front)] of 0.73 for T. arjuna and 0.71 for T. terrestris. This active fraction was isolated and injected to gas chromatography/mass spectrometry (GC/MS).

GRAPHICAL ABSTRACT



KEYWORDS: Chawki worm, Bombyx mori, Aspergillus niger, botanicals, in vitro studies.

1. INTRODUCTION

Silkworm being a highly domesticated insect is prone to various biotic and abiotic stresses, leading to drastic loss in silk yield. Silkworm is highly susceptible to various diseases which accounts for 30
- 40 per cent loss in cocoon yield (Rashmi Joshi and Raja, 2023). The infectious silkworm diseases are caused by pathogenic microorganisms such as virus, bacteria, fungus and protozoan. Aspergillosis is one of the serious fungal disease of silkworm causing cocoon crop loss to the tune of 21.36 per cent (Vineet Kumar *et al*., 2004). In India, the percentage of disease occurence ranged from 5.32% (Feb-March), 21.36% (July-Aug) and crop loss up to 1.60 – 13.04 Kg per 100 dfls (Harbant Singh *et al*., 2011). The early instars *i.e.,* first and second instar silkworm larvae are more susceptible and later stage silkworms are fairly resistant to this disease. High temperature and high relative humidity conditions maintained during young stages are reportedly contributing factors to greater disease incidence during young age (Govindan and Devaiah, 1995). The fungal disease Aspergillosis prevails during winter and rainy seasons (Kumar, V. and Lakshmi, R. 2023).

2. MATERIALS AND METHODS

Experiments were conducted during 2023-2025 in the Sericulture Unit, Department of Entomology, SRM College of Agricultural Sciences, Baburayenpettai, Chengalpattu District, Tamil Nadu, India.

2.1. Isolation of fungal pathogens

Infected Chawki worms collected from various parts of Tamil Nadu were first microscopically examined in order to confirm the presence of the Aspergillosis using the binocular research microscope (at 10x). After confirming for the presence of Aspergillosis spores, they were isolated in the laminar flow chamber under aseptic conditions following standard isolation method. The infected larvae showing typical symptoms were cut into small bits. Chawki worms infected with *Aspergillus* spp. were transferred to sterile petridishs containing Rose Bengal Agar medium under aseptic conditions. The inoculated petridishs were incubated under sterilized bell jars at room temperature (27±1ºC) and observed at regular intervals. A loop full of fungal culture developed on the bits on Rose Bengal Agar plates were taken on a glass slide and observed under the microscope for the presence of conidia and conidiophores. After confirming the spores of the cultures, they were purified by single spore isolation technique.

2.2. Single spore isolation

This was done in order to maintain the purity of the culture for future use. Ten milliliter of clear, filtered two per cent water agar was poured into sterilized Petridishs and allowed to solidify. Diluted spore suspension was prepared separately in sterile distilled water from a seven-day old culture. One milliliter of suspension was spread uniformly on water agar plates, the excess of which was aseptically drained off.

After 24 h of incubation at 27±1ºC, the plates were examined, so as to locate single isolated germinating conidium and marked with marker pen on the bottom surface of the plate. The portion of agar directly above the mark was cut and transferred to a Rose Bengal Agar slant in such a way that the conidium-bearing surface was in contact with agar surface and incubated at 27±1ºC. Slants were obtained for each isolate and their colony characteristics were studied (Kawakami, 1982, Harinatha Reddy, 2017).

2.3. Maintenance of the culture

The fungus was sub cultured on Rose Bengal Agar slants and allowed to grow for seven days at 27±1ºC and preserved at 4ºC, subcultured under aseptic conditions periodically (Kawakami, 1982, Harinatha Reddy, 2017).

2.4. Screening of botanicals against pathogen

An experiment was laid out using six botanicals possessing antifungal property. Hexane and chloroform extracts from leaves of these botanicals were prepared using standard procedures. These extracts were evaluated against *Aspergillus* spp. using standard procedures.

List of botanicals used in this study.

Sl. No.	Scientific name	Common name	Family
1	*Coleus forskohlii Lour.*	Marunthu koorkan	Labiataceae
2	*Cassia fistula L.*	Aavarai	Caesalpinaceae
3	*Tribulus terrestris L.*	Nernji mullu	Zygophyllaceae
4	*Terminalia arjuna Roxb*	Marutham	Combretaceae
5	*Lawsonia inermis Linn*	Maruthani	Lythracae
6	*Eucalyptus citriodora Hook.*	Eucalyptus	Myrtaceae

2.5. Preparation of plant extract for screening

Leaf extracts were prepared from plants and its antifungal activity was tested against *Aspergillus* Spp. Extraction was done using Hexane and Chloroform (40-60 ºC) (Ankita and Kanika Sharma, 2011).

2.6. Drying of plant parts and preparation of the plant extracts

The leaves of plants *viz. C. forskohlii*, *C. fistula*, *T. terrestis*, *T. arjuna*, *L. inermis* and *E. citriodora* were air dried at room temperature for two weeks. The dried plant leaves were ground into fine powder form and packaged into polythene bags for future use. 25g of the powdered leaves of *T. terrestris, C. fistula, L. inermis, C. forskohlii, E. citridora* and *T. arjuna* were weighed separately into 240ml hexane or chloroform and percolated for 24 h. Soxhlet extraction instrument was used in the extraction procedure. The sample tube of the unit was fitted with a filter disc at the bottom and filled with ground samples, sealed with another filter disc and compressed. This was fitted to electric heating mantle with soxhlet unit, filled with 240ml of hexane or chloroform, and temperature of 40 ºC and 60 ºC was maintained for hexane and chloroform, respectively. The unit was regulated with water to give a slow controlled flow of the solvent through the compressed sample. The filtrate was collected in a rained bottom flask. The residual extract was collected in a flask and transferred to a rotary flash vacuum evaporator for evaporation of the solvent. The residue thus obtained was stored at 4^0C in airtight bottles for future use

(Tajamul Islam and Jasmeena Qadir. 2024). The percent extractive values were calculated by using the formula (Ankita and Kanika Sharma, 2011)

$$\text{Percent extractive} = \frac{\text{Weight of the dried extract}}{\text{Weight of dried plant material}} \times 100$$

2.7. Management of disease

Aspergillus disease is very serious in nature leading to severe loss in cocoon production. The efficacy of various plant extracts was tested against Aspergillosis at Chawki stage with the following treatments.

2.8. In vitro testing fungi toxicity of plant extracts

Agar well diffusion test was conducted for testing the efficacy of plant extracts against *Aspergillus*. Six mm of *A. niger* disc was placed in the center of the Petridish (90 mm in diameter) containing Rose Bengal Agar medium. Agar wells (6 mm in diameter) were laid on the agar surface at 1 cm away from the periphery of Petri dish and 100 µl of the plant extract was poured in three wells at 2000ppm, 3000ppm and 4000ppm concentrations respectively. One petridish (90mm) was considered as a check. For untreated check three wells were made in petridish, and 100 µl of hexane and chloroform were poured into two wells and the third well was maintained without any solvent. A disc with *A. niger* was placed at the centre of the plate. The plates were incubated at room temperature and inhibition zone was recorded on seventh day (Tajamul Islam and Jasmeena Qadir. 2024).

In vitro treatments for A. niger

Treatments	Botanicals	Concentrations (ppm)
T1	*Tribulus terrestris*	2000/ 3000/ 4000
T2	*Coleus forskohlii*	2000/ 3000/ 4000
T3	*Eucalyptus citridora*	2000/ 3000/ 4000
T4	*Cassia fistula*	2000/ 3000/ 4000
T5	*Lawsonia inermis*	2000/ 3000/ 4000
T6	*Terminalia arjuna*	2000/ 3000/ 4000
T7	Hexane alone	
T8	Chloroform alone	
T9	Control (without solvent)	

2.9. Separation of antifungal compounds through Thin Layer Chromatography (TLC)

2.9.1. Preparation and activation of TLC plates

Silica gel – G (E –Merck) was used for preparing TLC plate of dimension 20 X 20 cm. Twenty-five gram of finely powdered silica gel was mixed thoroughly with 40 ml of distilled water. The slurry was poured into TLC applicator, which was adjusted for 0.5 mm thick wet silica gel. The glass plate was allowed to dry in open air for 1h and then heated in hot air oven at 110ºC for 2 h. The activated plate was loaded with 20 µl of the sample using a capillary tube without disturbing the silica gel layer (Alejandro *et al*., 2008)

2.9.2. Preparation of sample for TLC

After evaporation the left over botanical residue of chloroform extraction was dissolved in 10 ml of acetone (Alejandro *et al*., 2008).

2.9.3. Separation of compounds through TLC

Using the capillary tube, 20 µl of the sample was applied on the activated plates and run separately for

90 min in the solvent system of chloroform: methanol: glacial acetic acid: ethyl acetate (50:40:05:05). Compounds were detected by spraying Folin–Ciocalteau reagent 1N) followed by spraying 20 per cent Na_2CO_3 solution. Folin–Ciocalteau reagent was used to identify compounds. Presence of compound is indicated by specific colour spots (Alejandro *et al*., 2008). The TLC plate was sprayed with the revealing agent in order to identify the compound present in the leaf extracts. The TLC plate was dried and observed for the presence of coloured spots, which indicated the presence of phenolics, terpenes, alkaloids or flavonoids. All the spots were observed under UV light. The relation to front (Rf) of the spots developed on the TLC plate were recorded using the formula given below.

$$\text{Rf value} = \frac{\text{Distance (cm) spot travelled}}{\text{Distance (cm) solvent travelled}}$$

2.10. Antifungal activity of purified compounds by inhibition zone technique

To test the antifungal, the activity of purified compounds, 9 mm of fungal disc was placed in the center of the Petri dishes (90 mm in diameter) containing the Rose Bengal Agar medium and the plates were incubated for 72 h at room temperature (28 ± 2 ºC). After 72 h, sterile filter paper discs (6 mm in diameter) were laid on the agar surface at 1 cm away from the periphery of Petri dish and 100 µl of the purified compounds (separated from TLC) were applied on each disc. Acetone applied on another disc was considered as check. The plates were incubated at room temperature and inhibition zone was recorded at 72 h after the onset of the treatment (Mauch *et al*., 1988).

2.11. Gas Chromatography – Mass Spectrometer analysis (GCMS):

GC–MS was performed by a THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II. The coloum used was DB 5 - MS CAPILLARY STANDARD NON - POLAR COLUMN. In this system, the thermo stated block of TDS2, which contains a tube, formed the extraction unit. A capillary transfer line connected this block with the GC injector, which can be cooled down to −10ºC (with nitrogen) and heated up to 300ºC. Helium flowed through the tube and the transfer line to the injector. The carrier gas flow (Helium) favoured the stripping of volatiles, which were then trapped and focused on the cooled surface of the injector liner. Volatile compounds were transferred to the cooled injector in split less mode. The control of the numerous operating parameters of the extraction unit was obtained by an external automatic controller (Mega Sharma *et al*., 2002).

For the gas chromatographic-mass spectrometric analysis of the volatile compounds a 30 Mts, ID: 0.25 mm, FILM: 0.25µm DB-5MS fused-silica capillary columns (Agilent, USA) were used at a helium flow rate of 1 ml/min. The oven temperature was kept at 80^0C for 1 min and raised to 300^0C for 5min. The identification of compounds was obtained by comparison with Wiley 275 Mass Spectra Library.

2.12. Statistical analyses

The data collected in various experiments were statistically analysed using completely randomized design (CRD). The data were transformed to either Arc sine (Angular transformation) or Square root transformation for the purpose of analysis wherever needed. Duncan's Multiple Range Test (DMRT) was applied for comparing the treatment means (Gomez and Gomez, 1983).

3. RESULTS AND DISCUSSION

The yield of extracts differed considerable between plant species and solvents (Table 1 and Fig 1) The chloroform solvent had a remarkably greater yield compared to hexane, thus illustrating its greater efficiency in extracting relatively polar secondary metabolites. The highest yield of chloroform extracts was obtained in *Eucalyptus citriodora* (40.80%) and *Coleus forskohlii* (38.80%), thus depicting a relatively greater presence of chloroform-soluble fractions and their bioactive classes in this species and its high solubility with chloroform solvent. The moderate yield was obtained in *Lawsonia inermis* (6.40%) and *Cassia fistula* (4.40%), while a low yield value was recorded in *Tribulus terrestris* (3.20%) and *Terminalia arjuna* (1.60%).

Table 1: Per cent Extractive Value (Total Yield) of Selected Medicinal Plants UsingDifferent Solvents

Sl. No.	Solvents	*T. errestris*	*T. arjuna*	*C. fistula*	*C. forskohlii*	*L. inermis*	*E. citridora*
1.	Chloroform	3.20	1.60	4.40	38.80	6.40	40.80
2.	Hexane	2.80	3.60	3.60	4.00	3.20	8.80

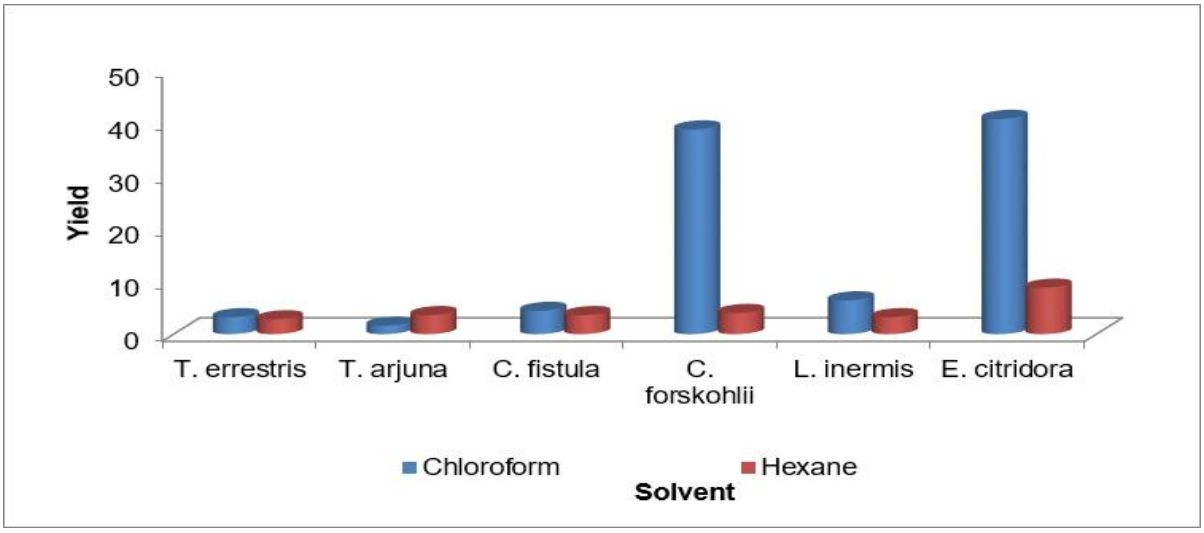


Figure 1: Per cent extractive value (Total yield)

Hexane extracts gave relatively low results for every species, with *E. citriodora* producing the highest purity of 8.80%, marking its less significant presence of non-polar phytochemicals like lipids and waxes. These results thus highlight the importance of the polarity of solvents applied in phytochemical extraction processes for discovering better leads from *E. citriodora* and *C. forskohlii*.

An experiment was laid out to find out the efficacy of botanical leaf extracts (hexane and chloroform extracts) *viz., T. terrestris, C. forskohlii, E. citridora, C. fistula, L. inermis and T. arjuna* at different concentrations of 2000ppm, 3000ppm and 4000ppm were tested against *A. niger* in *in vitro* condition. Six mm of *A. niger* disc were placed in the center of the Petridishs (90 mm in diameter) containing Rose Bengal Agar medium. Agar wells (6 mm in diameter) were laid on the agar surface at 1 cm away from the periphery of Petri dish and 100 µl of the plant extract was poured in three wells at 2000ppm, 3000ppm and 4000ppm concentrations respectively. One petridish (90mm) was considered as a check. For untreated check three wells were made in petridish, 100 µl of hexane and chloroform were poured into two wells and the third well was maintained without any solvent. The plates were incubated at room temperature and inhibition zone was recorded on seventh day of the experiment.

The results of evaluation of botanicals (hexane extract) at different concentrations *viz.,* 2000ppm, 3000ppm and 4000ppm against *A. niger* inhibiting zones are presented in Table 2 and Fig 2. The hexane extract of the *T. arjuna* gave maximum inhibition zone of 19.8, 21.0 and 22.8mm at 2000, 3000 and 4000ppm respectively. This was followed by *C. fistula* (19.0mm, 20.1mm and 21.0mm) and *T. terrestris* (15.1mm, 16.2mm, 17.8mm). Whereas *C. forskohlii* treatment registered 7.1, 9.0 and 11.1mm. Hexane and control treatments recorded inhibited zone of 0.6 and 0 mm respectively on *A. niger*.

Table 2: Antifungal activity of botanical leaf extracts (Hexane extract) against A. niger

Sl. No.	Botanicals	Diameter of inhibition zone (mm)		
		2000 ppm	3000 ppm	4000 ppm
1	*Tribulus terrestris*	15.1 ^{b} (4.5)	16.2 ^{b} (4.6)	17.8 ^{b} (4.8)
2	*Coleus forskohlii*	7.1 ^{e} (3.5)	9.0 ^{e} (3.7)	11.1 ^{e} (4.0)
3	*Eucalyptus citridora*	11.0 ^{d} (4.0)	13.0 ^{d} (4.2)	14.3 ^{d} (4.4)
4	*Cassia fistula*	19.0 ^{a} (4.9)	20.1 ^{a} (5.0)	21.0 ^{a} (5.1)
5	*Lawsonia inermis*	12.2 ^{c} (4.1)	15.0 ^{c} (4.5)	17.3 ^{c} (4.7)
6	*Terminalia arjuna*	19.8 ^{a} (5.0)	21.0 ^{a} (5.1)	22.8 ^{a} (5.3)
7	Hexane alone	0.6 ^{f} (2.4)	0.6 ^{f} (2.4)	0.6 ^{f} (2.4)
8	Control	0.0 ^{f} (2.2)	0.0 ^{g} (2.2)	0.0 ^{f} (2.2)

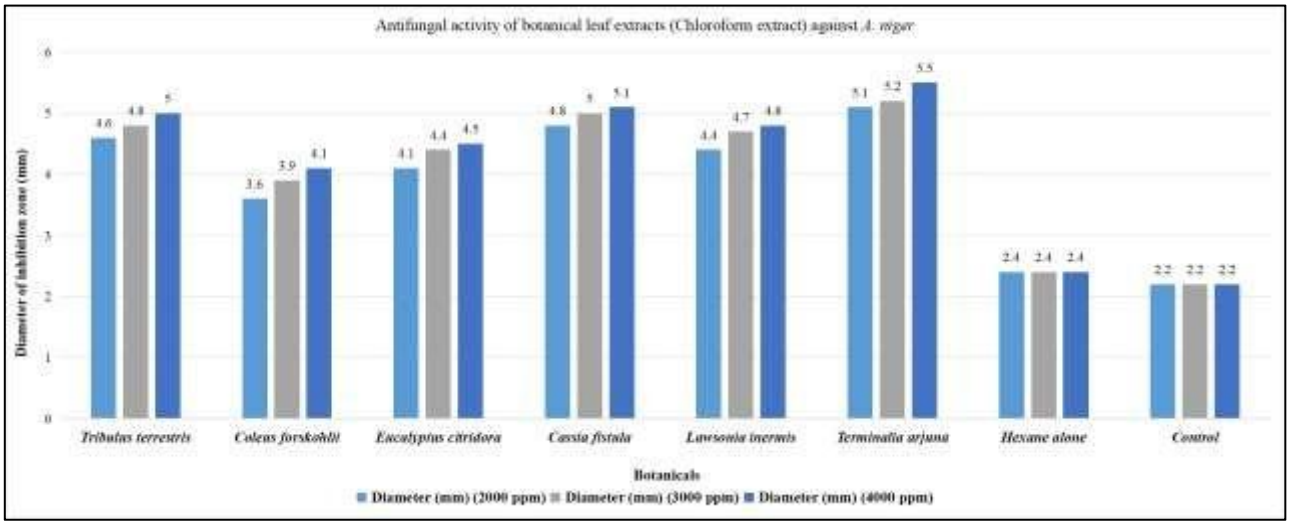


Figure 2: Anti-fungal activity of botanical leaf extracts (Chloroform extract) against Aspergillus niger

The results of evaluation of botanicals (chloroform extracts) at different concentrations *viz.,* 2000ppm, 3000ppm and 4000ppm against *A. niger* inhibiting zones (Table 3 and Fig 3). The chloroform extract of the *T. arjuna* were found to have maximum inhibition zone of 20.8, 21.9 and 25mm at 2000, 3000 and 4000ppm concentrations respectively. This was followed by *C. fistula* (17.6, 20.1and 21.9 mm) and *T. terrestris* (16.1, 17.9 and 20.1mm). Whereas *C. forskohlii* treatment registered an inhibition zone of 8.2, 9.9 and 12.1mm at 2000, 3000 and 4000ppm concentrations respectively. The inhibition zone of 1.0 and 0.0 mm were recorded in chloroform alone and control was respectively.

Table 3: Antifungal activity of botanical leaf (Chloroform extract) against A. niger

Sl. No.	Botanicals	Diameter of inhibition zone (mm)		
		2000 ppm	3000 ppm	4000 ppm
1	*Tribulus terrestris*	16.1 $_{c}$ (4.6)	17.9 $_{c}$ (4.8)	20.1 $_{b}$ (5.0)
2	*Coleus forskohlii*	8.2 $_{f}$ (3.6)	9.9 $_{e}$ (3.9)	12.1 $_{e}$ (4.1)
3	*Eucalyptus citridora*	12.0 $_{e}$ (4.1)	14.1 $_{d}$ (4.4)	15.2 $_{d}$ (4.5)
4	*Cassia fistula*	17.6 $_{b}$ (4.8)	20.1 $_{b}$ (5.0)	21.9 $_{b}$ (5.1)
5	*Lawsonia inermis*	14.2 $_{d}$ (4.4)	17.2 $_{c}$ (4.7)	18.3 $_{c}$ (4.8)
6	*Terminalia arjuna*	20.8 $_{a}$ (5.1)	21.9 $_{a}$ (5.2)	25.0 $_{a}$ (5.5)
7	Hexane alone	1.0 $_{g}$ (2.4)	1.0 $_{f}$ (2.4)	1.0 $_{f}$ (2.4)
8	Control	0.0 $_{h}$ (2.2)	0.0 $_{g}$ (2.2)	0.0 $_{g}$ (2.2)

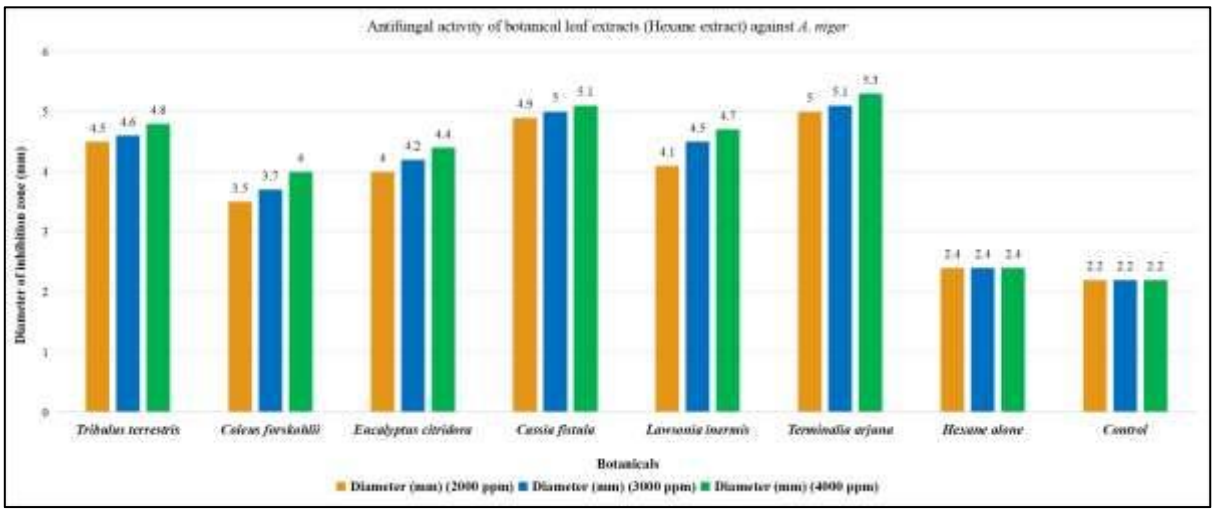


Figure 3: Anti-fungal activity of botanical leaf extracts (Hexane extract) against Aspergillus niger

In vitro studies on antifungal activity of botanicals against *A. niger* revealed that 4000 ppm of chloroform and hexane extracts of *T. arjuna, C. fistula* and *T. terrestris* showed significant reduction in fungal growth. Similar results were obtained by Dickson Thomas Manyama *et al*.,2023 evaluated the in-vitro antifungal efficacy of five locally available botanicals (Azadirachta indica, Calotropis procera, Euphorbia hirta, Jatropha curcas, and Ricinus communis) against the growth of *A. niger*. However, Thapliyal *et al*., (2000) reported that crude protein extracts of medicinal plants (*Rauwolfia tetraplylla L*. and *Andrographis paniculata* tested against Aspergillus for their toxicity and found that 100 per cent inhibition was observed at 66, 48 and 30 µg. Plant extracts are known to inhibit the growth of several pathogenic fungi (Pandey *et al*., 1981; Singh and Tripathi, 1993).

In concurrence with present studies, (Said I. Behiry *et al*., 2022) different organic solvent extracts of turmeric, wheat bran, and taro peels, 75% ethanol extract of taro was extremely active against *Aspergillus niger* growth. In line with present investigations, Singh *et al*. (2002) reported that *in vitro* studies using henna leaf and mango bark at 1.0, 2.0 and 3.0 per cent concentrations against *A. niger* and *A. tamarii* revealed that they exhibited considerable effect on *Aspergillus* infection in silkworm.

3.1. Thin layer chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS)

Based on the *in vitro* and *in vivo* studies on evaluating the efficacy of botanicals against *A. niger,* two botanicals *viz.,* chloroform leaf extracts of *T. arjuna* and *T. terrestris* showed maximum antifungal activity when compared to other plant leaf extracts against *A. niger*. These two plants were subjected to TLC method for separation of active compounds. The observations after 4 h revealed that three spots of good resolution with Rf values of 0.06, 0.2, 0.73 for *T. arjuna* and 0.13, 0.16, 0.71 for *T. terrestris* were observed on the chromatogram.

The spots identified at 0.73 and 0.71 (Rf values) resulted during bioautography of chloroform extracts of *T. arjuna* and *T. terrestris.* These were isolated by column chromatography with chloroform and on estimating their antifungal activity resulted in maximum zone of inhibition by paper disc method. These active fractions were isolated and injected to gas chromatography/mass spectrometry (GC/MS) for identifying the actual active compounds in the particular spot.

The results after operating in the GC-MS instrument provide the active compounds present in *T. arjuna* and *T. terrestris*. These active compounds are:

1. (E)-17á-[[(4-Dimethylaminobutoxy) imino] methyl]-5á-androstane-3á,14á-diol oxalate,
2. 5-(2-Bromotetrafluoroethyl)-5-hydroxy-3-methyl-4,5-dihydroisoxazole,
3. 1-(Dimethylamino)-2-butene-2,3-dicarbonitrile,
4. 1-Pentyl-1,3-dihydrobenzo[c]furan,
5. (1R,3S)-(+)-N-(3-Ethenyl-2,2-dimethylcyclobutyl)acetamide,
6. 2-(Dimethylaminomethyl)-1-methylenecyclooctane
7. o-Deuteriobenzaldehyde Tosylhydrazone.

3.2. Compounds having Antifungal properties

1) (E)-17á-[[(4-Dimethylaminobutoxy) imino]methyl]-5á-androstane-3á,14á-diol oxalate
Androstane are steroid compounds having specific antifungal activities. Specific synthetic modifications of fatty acid esters has the antifungal a activities. They are reported to be toxic to Candida albicans, Cryptococcus neoformans, Candida glabrata, and the filamentous fungus Aspergillus fumigatus. Several of the derivatives from the androstane groups showed reasonable antifungal activity (as measured spectroscopically by a >25% reduction in fungal growth compared to control wells) against at least one species of fungus (Jursic et al, 2010).
2) 5-(2-Bromotetrafluoroethyl)-5-hydroxy-3-methyl-4,5-dihydroisoxazole
Isoxazolines, isosters of oxazolidinones, which are well-known antibacterial agents, have recently been reported to be of great significance as antibacterial and antifungal agents. Several derivatives of these compounds were tested for the antifungal activities. It were screened against Candida albicans, Candida parapsilosis, Cryptococcus neoformans, Aspergillus fumigatus, Sporothrix schenckii, and Trychophyton mentagrophytes. These derivatives of isoxazolines exhibited inhibition of fungal growth at a concentration ranging from 50 mg/mL to 6.25 mg/Ml (Mishra et al., 2004).
3) 1-(Dimethylamino)-2-butene-2,3-dicarbonitrile
Dicarbonitriles are organic compounds having benzene ring with nitrogen bonded. In a report by (Nosachev et al, 2010) a series of 3-aryl-4,4(5H)-dicarbonitrile-5-phenyloxazolines have been synthesized and tested for activity against Candida albicans, Microsporum canis, and Trichophyton rubrum fungal species. The degree of sensitivity of the studied microorganisms to the compounds was determined visually from the area of growth inhibition around the drug carrier (fungistatic activity) or the 50% growth suppression of the microorganisms (fungicidal

activity). These compounds had low toxicity and exhibited various antifungal activity.

4) 1-Pentyl-1,3-dihydrobenzo[c]furan
Furan are heterocyclic compound has the chemical formula (CH2)4O. These isomers wre sreend against displayed Candida as well as Aspergillus. It showed improved activity over both itraconazole and saperconazole. The tetrahydrofuran compound shows an in vitro spectrum and potency comparable to itraconazole, but is not as potent overall as its regioisomer (Lovey et al ,2007).

5) (1R,3S)-(+)-N-(3-Ethenyl-2,2-dimethylcyclobutyl)acetamide
Derivatives of acetamides were extracted from the fermented broth of Streptomyces sp. Antifungal property of the 4' phenyl-1-napthyl-phenyl acetamide was determined by agar diffusion assay method using the pathogens such as Aspergillus flavus, A. niger, A. fumigatus, Mucor sp. Penicillium sp and Candida albicans. The in vitro antifungal activity of 4' phenyl-1-napthyl-phenyl acetamide showed maximum inhibitory activity against Candida albicans (25.05±0.81) followed by Aspergillus niger , A. flavus (13.6±1.23), Mucor (13.27±0.44), A.
fumigatus (10.8±0.49), and minimum inhibitory activity was observed with and Penicilliumsp (Dhanasekeran et al ., 2008)

6) 2-(Dimethylaminomethyl)-1-methylenecyclooctane
The versatile (dimethyl amino methylene) cyclooctanone was used as a key intermediate for the synthesis of cyclooctanones and cyclooctane-based heterocycles with pyrazole, isoxazole, pyrimidine, pyrazolopyrimidine, triazolopyrimidine and imidazopyrimidine derivatives via its reactions with several nitrogen nucleophiles. These synthesized compounds were screened in vitro for their antimicrobial activity against pathogenic microorganisms (Listeria monocytogenes, methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans). Most of the tested compounds showed moderate to high antibacterial and antifungal effects against the tested pathogenic microorganisms. Among the synthesized compounds, 2-((p-sulfonamidophenyl)methylene)cyclooctanone showed excellent activity against Listeria monocytogene (Hosni et al ., 2007).

7) o-Deuteriobenzaldehyde Tosylhydrazone
A series of hydrazones synthesized from various cholesterol derivatives were evaluated for their in vitro antimicrobial properties against human pathogens. The activity was highly dependent on the structure of the different compounds involved. The best results have been obtained with tosylhydrazone cholesterol derivatives exhibiting activities against Candida albicans (CIP 1663-80) at a concentration of 1.5 µg/mL (Loncle et al., 2004)

4. CONCLUSION

Present study was taken up to survey for *Aspergillosis* in chawki worms in CRCs available in various districts of Tamil Nadu and to find out botanicals for management of this disease-causing great loss to chawki rearing. *In vitro* studies on antifungal activity of botanicals against *A. niger* revealed that 4000 ppm of chloroform and hexane extracts of *T. arjuna*, *C. fistula* and *T. terrestris* showed significant reduction in fungal growth.

- Bioautography of chloroform extracts resulted in *T. arjuna* and *T. terrestris* identification of active compound at 0.73 and 0.71 Rf values. This was isolated by column chromatography with chloroform and this showed maximum zone of inhibition by paper disc method.
- This active fraction was isolated and preparative by TLC and controlled by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).
- The GC-MS instruments indicating the Anti-fungal compounds both *T. arjuna* and *T. terrestris viz.,*

✔ (E)-17á-[[(4-Dimethylaminobutoxy)imino]methyl]-5á-androstane-3á,14á-diol oxalate,

✔ 5-(2-Bromotetrafluoroethyl)-5-hydroxy-3-methyl-4,5-dihydroisoxazole,

✔ 1-(Dimethylamino)-2-butene-2,3-dicarbonitrile,

✔ 1-Pentyl-1,3-dihydrobenzo[c]furan,

✔ (1R,3S)-(+)-N-(3-Ethenyl-2,2-dimethylcyclobutyl)acetamide,

✔ 2-(Dimethylaminomethyl)-1-methylenecyclooctane,

✔ o-Deuteriobenzaldehyde Tosylhydrazone.

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