

## Identification of Antifungal Compounds from *Solanum torvum* Against Post Harvest Anthracnose of Banana

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### ABSTRACT

The fungitoxic effect of 25 botanicals belonging to 17 different families was evaluated against *Colletotrichum musae* (Berk. & M. A. Curtis) Arx causing banana anthracnose disease. Among all, *Solanum torvum* showed the maximum antifungal activity followed by the *Allium sativum* × *Allium cepa* (Zimmu) and *Adenocalymma alliaceum*. Post-harvest treatment of banana fruits with leaf extracts of *S. torvum*, zimmu, and *A. alliaceum* in room and cold storage condition significantly reduced the disease incidence and also *de novo* synthesis of defense related Peroxidase (PO) and PolyPhenol Oxidase (PPO) compounds. Of all the leaf extracts, *S. torvum* showed the least incidence of disease with highest activity of defense related compounds. An attempt was made to identify the antifungal compounds through chromatographic techniques like TLC and RP-HPLC. Different leaf extracts of *S. torvum* were obtained using different solvents and evaluated fungitoxic effect against the pathogen *in vitro*. Acetone was found the best in extracting the bioactive antifungal compounds from *S. torvum*. Further TLC analysis showed the presence of phenolic compound and development of blue spot at R<sub>f</sub> 0.65 and eluted purified blue spot exhibited a maximum inhibition of the pathogen *in vitro*. The presence of syringic acid was evident from the RP-HPLC analysis. The results seem to be the new report for the presence of phenolic compounds in the leaf extract of *S. torvum*. The botanical *Solanum torvum* could be used as botanical fungicide, as an alternative to synthetic fungicides to manage the disease effectively.

**Keywords:** Anthracnose, Botanicals, Phenolics, Plantain, TLC and HPLC.

### INTRODUCTION

Banana (*Musa* spp.) is the most important fruit crop for small-scale farmers in tropical and subtropical regions of India and it is cultivated in about 0.85 million ha with an annual production of 29.2 million tons (IndiaStat, 2017). The plantain is prone to the attack by several diseases, among which panama wilt, sigatoka leaf spot, anthracnose, banana bunchy top, banana streak mosaic and banana bract mosaic are predominant. Among others, anthracnose caused by *Colletotrichum musae* (Berk. & M. A. Curtis) Arx occurs in almost all the banana growing regions. Losses due to anthracnose

disease are estimated about 20 to 25% of harvested fruits during post-harvest handling even in developed countries (El-Ghaouth *et al.*, 2004; Zhu, 2006; Singh and Sharma, 2007). However, in developing countries, post-harvest losses are often more severe due to inadequate storage and transportation facilities (Rashad *et al.* 2011).

The most commonly used fungicides for the management of banana anthracnose disease is benzimidazole fungicides, such as benomyl and thiabendazole (Jeger *et al.*, 1996; Khan *et al.*, 2001). However, *C. musae* has developed resistance to these fungicides and causing the residual toxicity of the chemicals (Eckert *et al.*, 1994; de Lapeyre and Chilin-Charles, 2008). The

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interest in finding alternative approaches to control postharvest diseases that fit well with the concept of sustainable agriculture has thus greatly increased (Mari *et al.*, 2007). It is therefore an utmost priority to identify alternative management strategy, particularly those which are environmentally safe and biodegradable, without sacrificing the productivity, thus, replacement of synthetic fungicides by natural products (particularly of plant origin). Botanicals have natural origin and are biodegradable and mostly do not leave toxic residues or byproducts to contaminate the environment.

Higher plants contain a wide spectrum of secondary substances such as phenols, flavonoids, quinones, tannins, essential oils, alkaloids, saponins, and sterols. Such plant chemicals may be exploited for their different biological properties, particularly for disease management (Wain, 1977; Mahadevan, 1982). Investigations on the mechanism of disease suppression by plant products have suggested that the active principles present in them may either act on pathogen directly or induce systemic resistance in host plants resulting in the reduction of disease development (Paul and Sharma, 2002). Keeping in view the above reasons, we aimed to study the effect of various botanicals on eco-friendly management of post harvest banana anthracnose disease.

## MATERIALS AND METHODS

### Fungal Culture

During the survey, the banana fruits showing characteristic symptoms of anthracnose were collected and brought to laboratory and pathogen was isolated by tissue segment method (Rangaswamy, 1958) using PDA medium. Identification of the fungal isolates was done using appropriate taxonomic key and description (Sutton, 1980, 1992). A colony produced from a single spore was maintained as a pure isolate throughout the study. The pure isolate was

sub cultured on PDA slants and allowed to grow for seven days at  $27\pm1^{\circ}\text{C}$  such slants were preserved in refrigerator at  $4^{\circ}\text{C}$  and sub cultured under aseptic conditions periodically.

### Plant Material

The twenty five botanicals including *Ageratum conizoides*, *Ocimum sanctum*, *Azadirachta indica*, *Allium sativum* A. cepa, *Ocimum basilicum*, *Plectranthus barbatus*, *Adenocalymma alliaceum*, *Catharanthus roseus*, *Datura metel*, *Eclipta alba*, *Eucalyptus lobules*, *Jatropha curcas*, *Lantana camara*, *Nerium odorum*, *Ocimum sanctum*, *Psoralea corylifolia*, *Bougainvillea spectabilis*, *Ricinus communis*, *Solanum torvum*, *Prosopis juliflora*, *Vitex negundo*, *Andrographis paniculata*, *Solanum trilobatum*, *Tridox procumbens* and *Aegle marmelos* belonging to the different families were collected from the botanical garden of Tamil Nadu Agricultural University, Coimbatore, India, and used throughout the study.

### Preparation of Leaf Extracts

The leaves of plants were collected and washed under tap water and then the leaves were rinsed with sterile distilled water. Ten grams of leaf material was weighed and triturated using a grinder by adding 10 mL sterile distilled water. The slurry was then filtered through a muslin cloth and centrifuged at 5,000 rpm for 10 minutes. Then, the supernatant was collected and filtered through bacterial filters to avoid bacterial contamination.

### Antifungal Activity of Leaf Extracts

Agar well diffusion test (Murray *et al.* 1995) was followed for testing the efficacy of the leaf extracts. Six mm of fungal disc was placed in the center of the Petri plates

containing the PDA medium, and agar wells (6 mm in diameter) were laid on the agar surface at 1 cm away from the periphery of the Petri plate, and 100  $\mu$ L of the leaf extracts were poured in each well. The Petri plate poured with distilled water was considered as a check. Five replicated plates were used for each leaf extracts. The plates were incubated at room temperature and the inhibition zone was recorded on the seventh day (Pandey *et al.* 1982).

### Leaf Extracts on Disease Incidence

Susceptible banana fruits variety Robusta free from visual damage was obtained and the fruits were washed in tap water to remove dust. Three effective leaf extracts (*S. torvum*, *A. sativum*  $\times$  *A. cepa*, and *A. alliaceum*) were selected and used for managing the disease under room ( $27 \pm 2^\circ\text{C}$ ), and cold conditions ( $10^\circ\text{C}$ ). Leaf extracts were prepared by grinding one kg of leaves in five liters of water, and dilution was made with water to obtain the final concentration of the extract as 10%. The fruits were dipped in the leaf extracts for ten min and then air dried for five hours. Fruits dipped in the sterile distilled water served as control.

Fruits were inoculated with pathogen using pin prick method. A circle of about five mm diameter was made with Indian ink, and injuries were made using a sterile needle in the marked area. The  $10^6$  conidial  $\text{mL}^{-1}$  suspension of *C. musae* was inoculated into the fruits separately. The inoculated fruits were kept inside sterile perforated polythene bags (200 gauge), which were sprayed with sterile distilled water so as to provide the required humidity. The fruits were kept incubated at room temperature and cold condition. Each polythene bag contained ten fruits, and each treatment had four replications. The Percentage of Disease Incidence (PDI) was calculated as the number of infected fruits showing symptoms of

anthracnose out of the total number of banana fruits.

### Activity of Defense Compounds

#### Activity Gel Electrophoresis

The changes in the induction of defense-related compounds in the inoculated fruits (positive control), inoculated fruits dipped in leaf extracts, and un-inoculated fruits (negative control) were analyzed at 24 hours of interval and up to seven days of post treatment.

#### Peroxidase (PO)

To study the expression pattern of different isoforms of PO in different treatments, activity gel electrophoresis was carried out. For native anionic polyacrylamide gel electrophoresis, resolving gel of 8% and stacking gel of 4% were prepared. After electrophoresis, the gels were incubated in the solution containing 0.15% benzidine in 6%  $\text{NH}_4\text{Cl}$  for 30 minutes in dark. Then, drops of 30%  $\text{H}_2\text{O}_2$  were added with constant shaking till the bands appeared. After staining, the gel was washed with distilled water and photographed (Sindhu *et al.*, 1984).

#### PolyPhenol Oxidase (PPO)

Enzyme was extracted by homogenizing one g of tissue in 0.01M potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at  $20,000 \times g$  for 15 minutes. at  $4^\circ\text{C}$  and the supernatant was used as enzyme source. After native electrophoresis, the gel was equilibrated for 30 minutes. in 0.1% p-phenylene diamine in 0.1M potassium phosphate buffer (pH 7.0) followed by 10 mM catechol in the same buffer. The addition of catechol was followed by a gentle shaking, which resulted



in appearance of dark brown discrete bands (Jayaraman and Ramanuja, 1987).

### Identification of Antifungal Compounds from *Solanum torvum*

#### Solvent Extracts of *S. torvum*

Leaf extraction was done using acetone, chloroform, hexane, methanol and petroleum ether with different concentrations viz., 5, 10, 15, 20, and 25%. Thirty mL of pure acetone was poured in a round bottom flask and 15 mL was poured in the funnel. The heating mantle was used to heat the round bottom flask. Temperature was maintained at 50°C. The evaporated acetone was condensed in the condenser and fell to the collecting funnel where the dried leaf powder was kept in a filter paper. Acetone slowly dissolved the polar components present in leaf material. When the collecting funnel filled up, the acetone moved down into the round bottom flask due to suction pressure. Thus all the soluble components of acetone accumulated in the round bottom flask. Soxhlet apparatus was allowed to run for 7 hours at 50°C. Chloroform, hexane, methanol and petroleum ether extracts were prepared by method described above, replacing acetone (40–60°C).

#### Efficacy of Solvent Extracts of *S. torvum* Against *C. musae*

Agar well diffusion test (Murray *et al.*, 1995) was followed for testing the efficacy of *S. torvum* extracts and inhibition zone was recorded at seventh day (Pandey *et al.*, 1982). Petri plate that was poured with respective solvent was considered as the control.

#### Thin Layer Chromatography

The crude aqueous extract of *S. torvum* was analyzed by TLC on Silica gel-G (E-

Merck), using TLC plate of dimension 20×20 cm. Twenty five grams 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 1 hours and then heated in hot air oven at 110°C for 2 hours. The activated plate was loaded with 20 µL of the sample using a capillary tube without disturbing the silica gel layer. Ten gram of *S. torvum* leaf was ground with 10 mL acetone and the extract was centrifuged at 11,200×g for 10 minutes and the supernatant was taken and evaporated to dryness in flash evaporator and the active principles were dissolved in 1 mL of acetone. Separation of phenolic compounds through TLC using the capillary tube 20 µL of the sample was applied on the activated plates and run separately for 90 minutes in the solvent system of chloroform: methanol: glacial acetic acid: ethyl acetate (50:40:05:05). Phenolic compounds were detected by spraying Folin–Ciocalteu reagent (1N) followed by spraying 20% Na<sub>2</sub>CO<sub>3</sub> solution (Sadasivam and Manickam, 1992; Nalina and Rahim, 2007). Presence of phenols was indicated by blue spots. The TLC plate was observed under UV light. The Relation to front (R<sub>f</sub>) of the spots developed on the TLC plate were recorded using the formula given below.

$$R_f \text{ value} = \frac{\text{Distance (cm) moved by the solute from the origin}}{\text{Distance (cm) moved by the solvent from the origin}}$$

#### Antifungal Activity of Purified Phenolics by Inhibition Zone Technique

To test the antifungal activity, 6 mm of fungal disc was placed in the center of the Petri plate containing the PDA medium and the plates were incubated for 72 hours at room temperature (28±2°C). After 72 hours, sterile filter paper discs (6 mm in diameter) were laid on the agar surface at 1 cm away from the periphery of Petri plate and 100 µL of the purified phenolic 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 hours after the onset of the treatment (Mauch *et al.*, 1988).

### Reverse Phase-High Pressure Liquid Chromatography (RP-HPLC)

Phenolic compounds in TLC purified retention factors were separated and identified using Shimadzu LC 8A RP-HPLC with C18 column. The pressure maximum was set to 300 psi with the flow rate of 1 mL min<sup>-1</sup>. UV wave length used for detection was 280 nm. Two pumps including A consisting of acetonitrile and B consisting of 0.1% phosphoric acid were used to run the TLC purified phenolic compounds. Gallic acid, Syringic acid, Catechol, Orcinol, Vanillic acid, Caffeic acid, Ferulic acid, Cinnamic acid, and Flavone were chosen as standards.

### Data Analyses

The data were statistically analyzed (Gomez and Gomez, 1984) using the SAS 9.2 version developed by the SAS institute, NC, USA. Lab experiments were carried out under completely randomized block design. The percentage values of the disease incidence were arcsine transformed. Data were subjected to Analysis Of Variance (ANOVA) at two significant levels ( $P < 0.05$  and  $P < 0.01$ ) and means were compared by Tukey's Honest Significant Difference (HSD).

## RESULTS

### Efficacy of Plant Extracts against *C. musae*

The antifungal activities of twenty-five plant extracts were evaluated *in vitro*. It was

observed that the inhibition zone varied from 0 mm to 25 mm (Table 1). The leaf extract of *S. torvum* recorded the maximum inhibition zone of 25 mm and completely checked the mycelial growth followed by *A. sativum* × *A. cepa* (17 mm) and *A. alliaceum* (16 mm), which were on par with each other. *Solanum trilobatum* and *Ageratum conizoides* showed an inhibition zone of 9 mm, whereas *Aegle marmelos* and *Jatropha curcus* showed only 3 mm inhibition zone. The other 18 leaf extracts were nil in inhibition zones. The most effective three plants were selected for further studies to manage the disease *in vivo*.

### Evaluation of Effective Leaf Extracts on Banana Anthracnose Disease under Room and Cold Condition

From the study, it was found that all three leaf extracts suppressed disease incidence to a greater extent in cold temperature than room temperature (Table 2). Under room temperature, the lowest PDI of 18.70 was recorded in banana fruits treated with *S. torvum*, followed by *Allium sativum* × *A. cepa* (26.03 %) and *A. alliaceum* (27.76 %). The highest PDI of 96.56 was recorded in inoculated control banana fruits alone whereas benomyl (0.1%) recorded the lowest PDI of 10.56. In the case of cold temperature, the lowest PDI of 13.43 was recorded in banana fruits treated with *S. torvum* followed by PDI of 20.46 and 23.60 in *Allium sativum* × *A. cepa* and *A. alliaceum*, respectively.

### Activity of Defense Related Compounds in Banana Fruits as Influenced by the Application of Leaf Extracts

#### Peroxidase

Native PAGE analysis of the enzyme extract from the banana fruit treated with *S. torvum*, *Allium sativum* × *A. cepa* and *A. alliaceum* indicated the expression of two isoforms *viz.*, PO1 and PO2. Among the

**Table 1.** Antifungal activity of different plant extracts on the mycelial growth of *C. musae*.

S No	Scientific name	Common Name	Family	Inhibition zone <sup>a</sup> (mm)
1	<i>Ageratum conizoides</i>	Floss flower	Asteraceae	9.0 <sup>c</sup>
2	<i>Ocimum sanctum</i>	Tulasi	Lamiaceae	0.0 <sup>e</sup>
3	<i>Azadirachta indica</i>	Neem	Meliaceae	0.0 <sup>e</sup>
4	<i>Allium sativum</i> × <i>A. cepa</i>	Zimmu	Alliaceae	17.0 <sup>b</sup>
5	<i>Ocimum basilicum</i>	Sweet basil	Lamiaceae	0.0 <sup>e</sup>
6	<i>Plectranthus barbatus</i>	Marunthu koorkan	Labiataceae	0.0 <sup>e</sup>
7	<i>Adenocalymma alliaceum</i>	Garlic creeper	Bignoniaceae	16.0 <sup>b</sup>
8	<i>Catharanthus roseus</i>	Red periwinkle	Apocynaceae	0.0 <sup>e</sup>
9	<i>Datura metel</i>	Oumathum	Solanaceae	0.0 <sup>e</sup>
10	<i>Eclipta alba</i>	Karisalankani	Compositae	0.0 <sup>e</sup>
11	<i>Eucalyptus lobules</i>	Eucalyptus	Myrtaceae	0.0 <sup>e</sup>
12	<i>Jatropha curcas</i>	Jatropha	Euphorbiaceae	3.0 <sup>d</sup>
e				
13	<i>Lantana camara</i>	Lantana	Verbenaceae	0.0 <sup>e</sup>
14	<i>Nerium odorum</i>	Arali	Apocynaceae	0.0 <sup>e</sup>
15	<i>Ocimum sanctum</i>	Thulasi	Labiataceae	0.0 <sup>e</sup>
16	<i>Psoralea corylifolia</i>	Karpogaarsi	Fabaceae	0.0 <sup>e</sup>
17	<i>Bougainvillea spectabilis</i>	Bougainvillea	Nyctaginaceae	0.0 <sup>e</sup>
18	<i>Ricinus communis</i>	Castor	Euphorbiaceae	0.0 <sup>e</sup>
e				
19	<i>Solanum torvum</i>	Turkeyberry	Solanaceae	25.0 <sup>a</sup>
20	<i>Prosopis juliflora</i>	Mesquite	Fabaceae	0.0 <sup>e</sup>
21	<i>Vitex negundo</i>	Notchi	Verbanaceae	0.0 <sup>e</sup>
22	<i>Andrographis paniculata</i>	Nilavembu	Acanthaceae	0.0 <sup>e</sup>
23	<i>Solanum trilobatum</i>	Purple-fruited pea	Solanaceae	9.0 <sup>c</sup>
Eggplant				
24	<i>Tridax procumbens</i>	Tridax daisy	Asteraceae	0.0 <sup>e</sup>
25	<i>Aegle marmelos</i>	Bael	Rutaceae	3.0 <sup>d</sup>
	Control	-	-	0.0 <sup>e</sup>

<sup>a</sup> Mean of three replications. Means followed by a common letter are not significantly different at 5 % level by Tukey's HSD.

**Table 2.** Efficacy of plant extracts on the incidence of banana anthracnose under room and cold condition. <sup>a</sup>

S No	Treatment	PDI at room condition			PDI at cold condition (10°C)		
		Days after inoculation			Days after inoculation		
		3	5	7	3	5	7
1	<i>S. torvum</i>	7.16 <sup>c</sup> (15.52)	14.13 <sup>c</sup> (22.08)	18.70 <sup>c</sup> (25.62)	4.25 <sup>c</sup> (9.09)	9.30 <sup>c</sup> (17.76)	13.43 <sup>c</sup> (21.49)
2	<i>Allium sativum</i> × <i>A. cepa</i>	9.45 <sup>d</sup> (17.90)	17.80 <sup>d</sup> (24.95)	26.03 <sup>d</sup> (30.67)	6.35 <sup>d</sup> (14.59)	14.18 <sup>d</sup> (22.12)	20.46 <sup>d</sup> (26.89)
3	<i>A. alliaceum</i>	10.86 <sup>e</sup> (19.24)	18.86 <sup>d</sup> (25.74)	27.76 <sup>d</sup> (31.79)	6.41 <sup>d</sup> (14.66)	16.56 <sup>e</sup> (24.01)	23.60 <sup>e</sup> (29.06)
4	Benomyl (0.1%)	4.10 <sup>b</sup> (11.66)	8.06 <sup>b</sup> (16.48)	13.23 <sup>b</sup> (21.32)	2.15 <sup>b</sup> (8.44)	5.16 <sup>b</sup> (13.12)	10.56 <sup>b</sup> (18.96)
5	Inoculated control	47.96 <sup>f</sup> (43.83)	79.53 <sup>e</sup> (63.10)	98.26 <sup>e</sup> (82.89)	40.38 <sup>e</sup> (39.45)	73.56 <sup>f</sup> (59.06)	96.56 <sup>f</sup> (79.33)
6	Uninoculated control	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>

<sup>a</sup> (a-f) Means followed by a common letter are not significantly different at 5 % level by Tukey's HSD. The values in parentheses are arcsine transformed; \*Mean of four replications.

two, the isoform PO2 was induced in all the leaf extracts treated banana, whereas isoform PO1 was expressed intensively in the *S. torvum* and *Allium sativum* × *A. cepa* treated banana fruit (Figure 1).

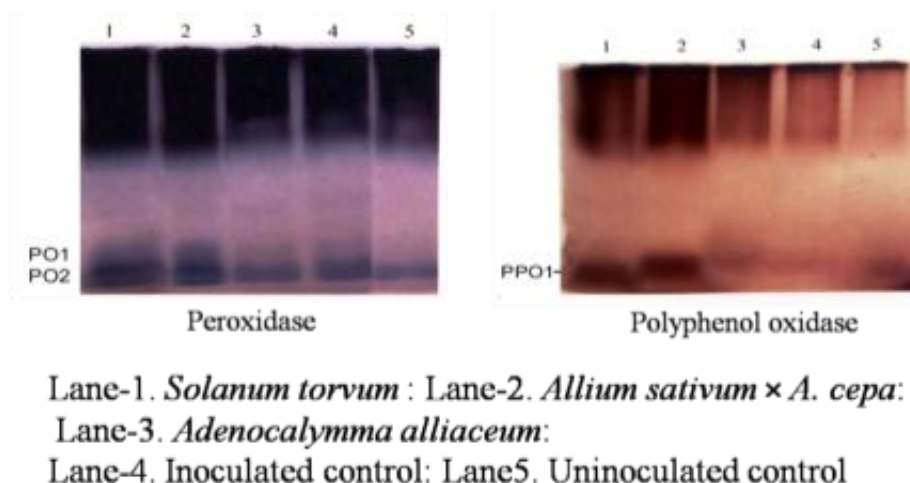
### Polyphenol Oxidase

Native PAGE analysis of the enzyme extract from the banana fruit treated with *S. torvum*, *Allium sativum* × *A. cepa* and *A. alliaceum*, indicated the expression of one isoforms of PPO1 (Figure 1).

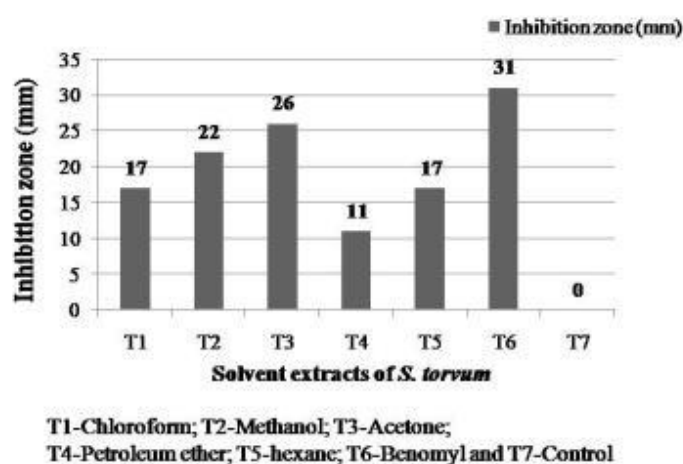
### Identification of Antifungal Compounds from *Solanum torvum*

#### Efficacy of Different Solvent Extracts of *S. torvum* Against *C. musae*

It was evident that acetone extract was effective in reducing the growth of the pathogen with 26 mm inhibition zone (Figure 2) followed by methanol (22 mm), chloroform (17 mm) and hexane (17 mm). Petroleum ether showed 11 mm inhibition zone, which was least effect against the pathogen. Among all solvents, acetone was



**Figure 1.** Expression of peroxidase and polyphenol oxidase isoforms in banana fruit in response to plant extracts.



**Figure 2.** Solvent extracts of *S. torvum* against mycelia growth of *C. musae*.



the best in extracting the bioactive antifungal compounds from *S. torvum*.

### Paration of Phenolic Compounds by Thin Layer Chromatography

The leaf extracts of *S. torvum* were subjected to thin layer chromatographic tests. The blue color spot was observed with Rf values of 0.65 (Figure 3) and indicated the presence of phenol. Presence of phenolics in *S. torvum* was also confirmed in readymade TLC. The separated phenolics appeared red in colour under UV-Spectrum (Figure 3).

### Antifungal Activity of the Purified Phenolic Compounds

The areas corresponding to the spots (Rf value of 0.65) were scrapped off, dissolved in acetone and subjected to antifungal assay, which showed a 67.78% inhibition over control (Table 3).

### Reverse Phase-High Pressure Liquid Chromatography (RP-HPLC)

The retention time for separation of phenolics using RP-HPLC varied with the individual phenolic compounds. In the study, the acetone extract of *S. torvum* was subjected to RP-HPLC for the separation of phenolic compounds. Results showed two peaks were observed with retention time of 5.056 minutes, which indicated the presence of syringic acid.

### DISCUSSION

Among the post-harvest diseases of banana, anthracnose caused by *C. musae* is found to be destructive. The losses may reach 100 per cent if these are not managed properly (Droby, 2006). It is a well-known fact that the use of chemical fungicides disturbs the ecosystem, induces the development of resistant mutant of the pathogen, and pollutes the environment. The direct application of fungicides on the surface of fruits may cause residual toxicity

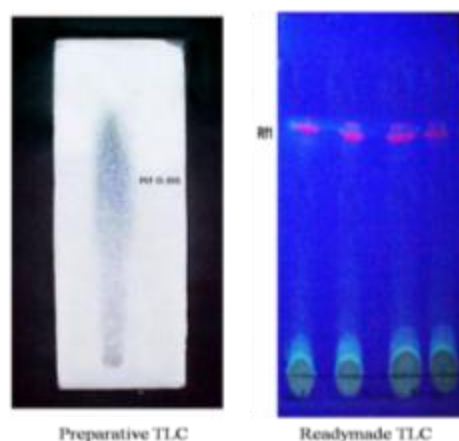


Figure 3. TLC analysis of phenolics in *S. torvum*.

Table 3. Antifungal activity of phenolic compounds isolated from *S. torvum* leaf extract by TLC.

Band color	Rf value	Radial growth (mm)	Percent inhibition over control
Blue	0.65	29.0	67.78
Control (Acetone)	-	90.0	0.0



also (De Lapeyre De Bellaire and Dubois, 1997). However, *C. musae* developed resistance to fungicides in addition to the residual toxicity of the chemicals (de Lapeyre and Chilin-Charles, 2008). Continuous and inappropriate use of chemical fungicides to manage anthracnose disease is not considered to be the long-term solution because this can increase the investment expenses, the risk of having high levels of toxic residues, and also the concerns in human health and environmental pollution (Latha *et al.*, 2009).

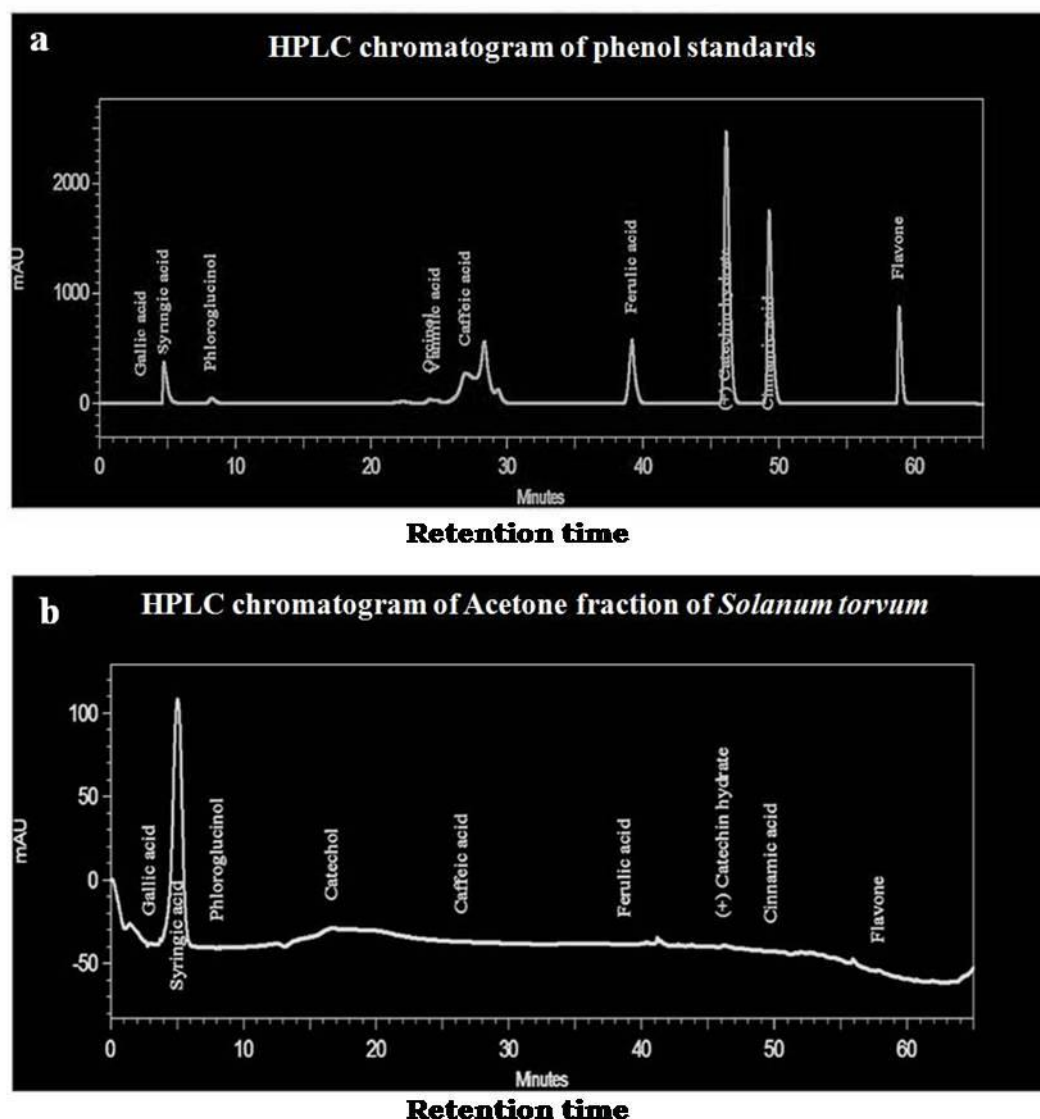
In the recent years, botanical fungicides are gaining momentum in the management of plant pathogens. Biocides of plant origin are non-phytotoxic, systemic and easily biodegradable. A number of plant species have been reported to possess natural substances that are toxic to a variety of plant pathogenic fungi (Spencer *et al.*, 1957; Fawcett and Spencer, 1970). The effectiveness of plant extract against pathogens might be due to the presence of antifungal constituents in the form of phenolic substances and gummy and non-volatile substances of unknown nature (Kosuge, 1969). The utilization of natural products, especially the plant extracts, has been shown to be effective against many plant pathogens and considered to be safe for consumers and environments (Hernandez-Albiter *et al.*, 2007).

With a view of the above context, an attempt was made to identify the effectiveness of leaf extracts against *C. musae*. Different botanicals were tested against the pathogen and disease development, among all *Solanum torvum*, *A. sativum*×*A. cepa* and *A. alliaceum* was found effective, and our results were in accordance with various researchers. Thangavelu *et al.* (2004) who reported that *S. torvum* (25%) inhibited mycelial growth of *C. musae*. Ogbabor *et al.* (2007) reported that *Allium sativum*, *Ocimum. basilicum* and *A. conyzoides* at 10% per cent totally inhibited the conidial germination of *C. gloeosporioides* in rubber. Rahman *et al.* (2011) indicated that *Jatropha curcas* had

the antifungal activity against *C. gloeosporioides* causing anthracnose of papaya. Richa *et al.* (2012) reported antifungal activity of Citrus Limon, *Persea americana* and *Carica papaya* against the *Colletotrichum gloeosporioides*. Plant extracts showing antimicrobial effect have been proved against several diseases (Anamika and Simon, 2011).

Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases (Gottlieb *et al.*, 2002). Bautista-Banos *et al.* (2002) reported effectiveness of various plant extracts in reducing the level of disease incidence of anthracnose of papaya and mango fruit caused by *Colletotrichum gloeosporioides*. Various plant extracts have been evaluated for their antifungal property against different pathogens and showed remarkable reduction of disease development (Tripathi and Dubey, 2004). Win *et al.* (2007) reported the efficacy of cinnamon extract treatment on crown rot disease of banana fruit. In the present study, extracts of *Solanum torvum*, *Allium sativum*×*A. cepa* and *Adenocalymma alliaceum* showed potential for reduction of anthracnose disease development on banana fruit. For the management of anthracnose disease in banana, exploitation of one or more of these botanicals is less expensive, safer for the applier and the ecosystem, and could serve as a good alternative to synthetic fungicides.

Activation of the plant's own defense system with the aid of biotic and abiotic inducer is a novel technology in the management of plant diseases. Plant products have been considered as one of the major groups of compounds that elicit the induced resistance. Biologically active compounds present in plant products act as elicitors and trigger the induced resistance in



**Figure 4.** Reverse Phase-High Pressure Liquid Chromatography (RP-HPLC) analysis of phenolic standards (a) and *Solanum torvum* (b).

host plants resulting in reduction of disease development (Deborah *et al.* 2001). In the present investigation, application of botanical extracts was studied for their induced resistance mechanism in terms of induction of defense related compounds viz., Peroxidase and Polyphenol oxidase. In the present study, banana fruits were treated with the plant extracts of *Solanum torvum*, *A. sativum* × *A. cepa* and *A. alliaceum* significantly reduced the anthracnose disease and also a successful induction of

resistance was mediated by de novo synthesis of Peroxidase and Polyphenol oxidase.

Several plant extracts are known to induce resistance in plants against various fungal and viral diseases (Lyon, 2007). Peroxidases are involved in phenyl propanoid metabolism, regulation of plant cell elongation, phenol oxidation, polysaccharide cross-linking, IAA oxidation, cross-linking of extensin monomers, oxidation of hydroxy-cinnamyl alcohols into free radical

intermediates and wound healing (Vidhyasekaran *et al.* 1997).

Polyphenol oxidase is a copper containing enzyme, which oxidizes phenolics to highly toxic quinones and involved in the terminal oxidation of diseased plant tissues and is attributed for its role in disease resistance (Kosuge, 1969). Anand *et al.* (2009) found that chili fruits inoculated with *C. capsici* and sprayed with leaf extract of *Abrus precatorius* recorded two-fold increase in Polyphenol oxidase activity. Aswini *et al.* (2010) also reported that mango fruits inoculated with the spore suspension of *C. gleosporides* and sprayed with 12.5% leaf extracts of *A. alliaceum* recorded three-fold increase in peroxidase and Polyphenol oxidase activity.

Extraction methods involve separation of antifungal compounds of plant tissue from inactive/inert components by using selective solvents. Among all solvents, acetone was the best to extract antifungal solvents from *S. torvum*. Our results were commensurate with the finding of Neela *et al.* (2014) reported acetone extract of nine leaf extracts showed antifungal activity against *Fusarium oxysporum* the causal agent of Fusarium wilt in tomato. Girijashankar and Thayumanavan (2005) reported that methanol extract of *Adenocalymma alliaceum* completely inhibited the growth of *P. aphanidermatum* and methanolic extract (10%) was effective against *M. phaseolina*. Satya *et al.* (2007) reported that methanolic leaf extract of Zimmu (*Allium sativum* × *Allium cepa*) showed effectiveness in inhibiting the growth of agriculturally important fungal and bacterial pathogens *viz.*, *Rhizoctonia solani*, *Aspergillus flavus*, *Curvularia lunata*, *Alternaria solani*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas campestris* pv. *malvacearum* and *Xanthomonas axonopodis* pv. *citri*.

Identification and characterization of fungal toxins of the tested plants will be helpful in effectively integrating the fungitoxic plant extracts in the integrated disease management strategies of various other crops. In the present investigation, the

antimicrobial compounds in *S. torvum* were identified and characterized by using the TLC and RP-HPLC techniques. The presence of phenolic compounds in *S. torvum* leaves was shown by means of R<sub>f</sub> value of 0.65 with blue spot. TLC purified phenolics of *S. torvum* were further identified using the RP-HPLC and compared with phenolic standards.

TLC purified phenolics of *S. torvum* were further separated and identified using RP-HPLC and compared with phenolic standards. RP-HPLC analysis revealed the presence of syringic acid in the leaf extract of *S. torvum* (Figure 4). There is no report available for the presence of phenolic compounds in the leaf extract of *S. torvum* and this seems to be the first report.

The antifungal nature of phenolic compounds present in botanicals has been documented by many researchers. Girijashankar and Thayumanavan (2005) reported antifungal activity of *Lawsonia inermis* against *Rhizoctonia solani*, *Pythium aphanidermatum* and *Macrophomina phaseolina* and identified the antifungal phenolic compounds such as tannic acid and catechol by RP-HPLC analysis. Similarly, Aswini *et al.* (2010) reported antifungal activity of *A. alliaceum* against *C. gleosporioides* causing mango anthracnose and identified the two phenolic compounds such as tannic acid and resorcinol in *A. alliaceum* by RP-HPLC analysis.

Akila *et al.* (2011) reported that presence of phenol compounds *viz.*, gallic acid, cinnamic acid, flavone, coumarin and syringic acid in *Datura metal* by RP-HPLC resulted in inhibition of mycelial growth of *F. oxysporum* f. sp. *cubense*. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987). The presence of phenols in plant product is considered to be potentially toxic to the growth and development of fungal



pathogens and thereby reduces plant diseases (Okwu and Okwu, 2004). The structural classes of phenolic compounds include the polyphenolic (hydrolysable and condensed tannins) and monomers such as ferulic and catechol (Okwu, 2005).

## CONCLUSIONS

In the present study, extracts from *S. torvum* showed potential for reduction of anthracnose development on banana fruit, indicating that biologically active phenolic compound like syringic acid could play significant role in crop protection strategies. Exploitation of naturally available chemicals from *S. torvum*, which retards the growth and reproduction of plant pathogens, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies. We can conclude that *Solanum torvum* could be used as possible bio-fungicide, as an alternative to synthetic fungicides against *Colletotrichum musae* on banana fruits to manage the anthracnose disease. Identification and characterization of the fungitoxic principles of *S. torvum* will be helpful in effectively integrating fungitoxic plant extracts in disease management strategies specific to various crops.

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## شناسایی مواد قارچ کش از *Solanum torvum* بر علیه مرض انتراکنوز بعد از

### برداشت موز

گ. جادشا، و پ. ولاپاگوندرا

### چکیده

در این پژوهش، اثرات سمی قارچ کش علیه *Colletotrichum musae* (Berk. & M. A. Curtis) Arx که موجب مرض انتراکنوز در موز می شود از ۲۵ گیاه دارویی متعلق به ۱۷ خانواده متفاوت بررسی شد. در میان همه، *Solanum torvum* بیشترین فعالیت ضد قارچی را نشان داد و بعد از آن *Adenocalymma alliaceum* و *Allium sativum* × *Allium cepa* (Zimmu) این بررسی، تیمار میوه های موز با عصاره برگ *S. torvum*، *A. alliaceum* و *zimmu* در شرایط اطاق و سردخانه به طور معناداری وقوع مرض را کم کرد و نو سازی مواد دفاعی پراکسیداز (PO) و پلی فنول اکسیداز (PPO) را کاهش داد. در میان همه عصاره های برگها، *S. torvum* کمترین مقدار وقوع مرض و بیشترین فعالیت مربوط به مواد دفاعی را نشان داد. نیز، تلاش شد تا مواد ضد قارچی با استفاده از تکنیک های کروماتوگرافی مانند TLC و RP-HPLC شناسایی شوند. عصاره های برگ های مختلف از *S. torvum* تهیه گردید و اثر مواد سمی قارچ کش بر علیه عامل بیماری در شرایط درونکشتگاهی (*in vitro*) ارزیابی شد. نتایج نشان داد که استون بهترین عصاره گیر مواد زیست فعال قارچ کش از *S. torvum* بود. تجزیه های بیشتر TLC حضور مواد فنولی را نشان داد و توسعه نقطه های آبی در Rf 0.65 و نقطه های آبی تصفیه شده شسته شده نشانگر بیشترین بازدارندگی عامل بیماری در شرایط درونکشتگاهی بود. تجزیه با RP-HPLC حضور اسید سرینگیک را نشان داد. این نتایج ظاهرا گزارش جدیدی است از حضور مواد فنولی در عصاره برگ *S. torvum*. برای مدیریت موثر این مرض، می توان گیاه دارویی *Solanum torvum* را به عنوان گیاه دارویی قارچ کش و جایگزین قارچ کش های صنعتی به کار بست.