PHYTOCHEMICAL CONSTITUENTS OF PENCIL TREE (*EUPHORBIA TIRUCALLI* L.) AS ANTIFUNGAL AGENT AGAINST MANGO ANTHRACNOSE DISEASE

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Abstract. The current study aimed to examine the *Euphorbia tirucalli* L. (pencil tree) *in vitro* antifungal activity against *Colletotrichum gloeosporioides* Penz. All the tested extract concentrations (1-3%) showed a significant decrease in the test fungus growth. However, 3% conc. exhibited the highest reduction in *C. gloeosporioides* biomass at 77%. Methanolic extract of *E. tirucalli* was examined through phytochemical analysis and determining flavonoids, saponins, terpenoids, tannins and alkaloids. The antioxidant activity of *E. tirucalli* was also tested using radical scavenging assay (DPPH), and 81% activity was observed at 200 µL conc. of methanol extract. Further solvent partitioning bioassay was employed to check the efficacy of the various isolated fractions viz. ethyl acetate, chloroform, *n*-hexane and *n*-butanol of the test plant against *C. gloeosporioides*. The bioassay results depicted that 0.5% and 1% concentration of the chloroform fraction of *E. tirucalli* caused the highest decline in the growth of *C. gloeosporioides*, i.e. 90% and 95%. This most effective extract was examined via GC-MS (gas chromatography-mass spectrometry). The major constituents identified were 9, 12-octadecadienoic acid (Z, Z)-, *n*-hexadecanoic acid, dodecanoic acid, tetradecanoic acid and phytol. Results suggest that active compounds detected by GC-MS analysis of *E. tirucalli* might be responsible for its antifungal potential.
Keywords: *antifungal bioassay, DPPH, flavonoids, phytochemicals, solvent partitioning bioassay*

Introduction

Mangifera indica L., commonly known as mango, is cultivated in the tropics and subtropical parts of the world (Sauco, 2017). It is the most popular commercially produced fruit crop. Pakistan, India, Brazil, Peru, Mexico, and the Philippines are the chief distributors of mango worldwide (Memon, 2016; Huang et al., 2018). Mango is an excellent source of fibre and powerful biochemicals such as carotenoids, provitamin A, vitamin C and phenolics. Owing to the availability of ample nutrients and impedance health benefits, fruits and vegetables have been of great importance in preventing various chronic diseases (Slavin and Lloyd, 2012; Schulze-Kayserset al., 2015; Shah et al., 2019).

*Colletotrichum gloeosporioide*s is one of the main plant pathogenic fungus responsible for anthracnose disease in mango. This fungus favours mild, humid environments for the constant and effective spread of anthracnose disease (Farr et al., 2006; Dean et al.,2012). Anthracnose disease dangerously deteriorates the mango crop, blooms with clear, deep, dusky brown to blackish spots on fruit before or after harvesting, and fruit may fall from trees prematurely (Pandey et al., 2012). At harvest, fruits that seem healthy may develop severe anthracnose symptoms once they ripen (Kenganalet al., 2010).

The successful approach against anthracnose disease is to employ post-harvest treatments with inorganic salts and hot-water dipping (Dessalegn et al., 2013).

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Various fungicides, such as copper hydroxide, azoxystrobin, mancozeb, prohloraz and copper sulfate products, are used to treat infection of *C. gloeosporioides* (Dirouet al., 2005; Sundravadanaet al., 2006). To improve resistance and development in the onset of disease, a lot of research has been conducted. However, the overproduction of precursors can overpower the disrupted biochemical pathway. Fungal cells can evolve mechanisms to prevent the fungicide from entering the cells and efficiently export the chemical out. The benzimidazole fungicides blocked the polymerization of tubulin and prevented the nuclear division of fungal cells. Simple natural mutations allowed for the creation of resistant fungi since the mode of action is so unique within the fungi. Therefore, resistance is very constant within the population and has developed due to the widespread use of benzimidazole fungicides (Pscheidt and Ocamb, 2018). Kumar et al. (2007) studied carbendazim (50 ppm), thiophanate-methyl (50 ppm), propiconazole (25 ppm), and hexaconazole (25 ppm) and two non-systemic fungicides, mancozeb (1000 ppm) and copper oxychloride (1000 ppm), to check the fungicidal resistance/sensitivity among six different isolates of *C. gloeosporioides* (Cg1-Cg7). Except for Cg3, which was mildly resistant to thiophanate-methyl, all isolates were highly susceptible to systemic fungicides. Cg1, Cg3, and Cg6 were highly vulnerable to mancozeb, while Cg5 and Cg7 were resistant, and Cg2 and Cg4 were highly resistant.

Generally, the systematic use of chemical fungicides for plant pathogen control has developed many related problems, including disease tolerance, human health damage, amassed toxic substances in plants and environmental pollution worldwide (Dubey et al., 2008). Using these substances creates awareness of potential risks to health and ecological consequences. Researchers have recently been interested in natural plant extracts due to their volatile nature, flavour, antioxidant, and antimicrobial effects (Dong et al., 2020; Topa et al., 2020). Plant secondary metabolism is responsible for producing alkaloids, terpenoids, flavonoids, polyphenols and phenols (Gulcin et al., 2004; Abad et al., 2007). These secondary metabolites/phytochemicals are reported as potent free radical scavengers and have significantly reduced the risk of diseases associated with oxidative stress (Wang et al., 2011; Wootton-Beard and Ryan, 2011). Many kinds of research are now focused on natural antioxidants as an alternative to synthetic additives in food and medicine. Hence, the alternative approach is to apply largely systematic, simply biological and non-phytotoxic botanical fungicides into the environment (Hanif et al., 2017; Munawwar et al., 2018; Bashir et al., 2019). There are vital records of rich antifungal components in the Euphorbiaceae family (Perea and Patterson, 2002; Silva et al.,2008; Khan et al., 2018).

Euphorbia tirucalli L. is a Euphorbiaceous plant commonly known as pencil tree, native to tropical regions with a pencil-like appearance. The white toxic latex secreted by this plant may contribute to its medicinal properties and low herbivore stress. The plant extract contains enormous amounts of sterols, terpenes and isolated substances, i.e. alfaeuforbol, taraxa, alcohol eufol, and tirucallol (Gupta et al., 2012). It was proposed from earlier literature that *E. tirucalli* possesses rich antimicrobic and pesticidal characteristics (Tiwari and Singh, 2006; Mwine and Van Damme, 2010; Jahanet al., 2011). Literature also supports that *E. tirucalli* extracts have an excellent antioxidant capacity and antimicrobial activity (Araujo et al., 2014). The latest interest is biologically sustainable antifungal compounds encouraged this research to study the antifungal efficacy of *E. tirucalli* against phytopathogenic fungal strain *C. gloeosporioides*.

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Materials and methods

Collection of experimental material

The whole plant of *E. tirucalli* had been collected from Canal Bank area Lahore, Pakistan. Before sterilizing, the products were washed thoroughly with tap water and treated with a sterilized distilled water solution containing one per cent sodium hypochlorite. Under sunlight, the plant materials were dried and ground to fine powder by an electrical blender and kept in zipper bags. The target fungus *C. gloeosporioides* was isolated from unhealthy mango inflorescence. The pathogen was identified using cultural, morphological, and conidial mechanisms such as colony colour, scale, and form. Mango fruit has previously been shown to have similar characteristics (Phoulivong et al., 2010; Wei et al., 2017). Sub-cultures were prepared and upheld on two per cent Malt Extract Agar (MEA) and maintained at 4 °C in the fridge.

In vitro bioassays

Assessment of test plant *E. tirucalli in vitro* was performed against target fungus (Hanif et al., 2017). Sun-dried fine grounded materials (50 g) of *E. tirucalli* were subjected to successive extraction with methanol (150 mL) and left for three days at 25 °C. Filtration through sterile muslin cloth was done, and the extract was concentrated using an electric oven at 40 °C. Extract production was 5.5 g and then reconstituted with 27.5 (mL) of sterilized distilled water to produce 20% of the solution in stocks. Different concentrations of *E. tirucalli* plant methanolic extract 1.0%, 1.5%, 2.0%, 2.5% and 3.0% v/v were prepared. Malt Extract (2%) medium, autoclaved in experimental flasks by adding ME (0.6 g) with 30 mL distilled water. Mixing of 1.5, 2.25, 3.0, 3.75 and 4.5 mL of stock solutions with 28.5, 27.75, 27, 26.25, 25.5 (mL) DH_2O in the medium was supplemented to make it up to 30 mL. No plant extract was added to the control flasks. To avoid bacterial contamination standard antibiotic as Amoxicillin (50 mg mL^{-1}) was added to each concentration. Each concentration was replicated thrice. C. gloeosporioides new discs were tipped in each flask for inoculation and left for incubation for seven days at 25 °C. The fungal growth was checked using filter paper (Whatman No. 1); after a week, dried the filtrate in an electronic oven, and weight was measured. Inhibition (%) was recorded by the given formula method:

$$\text{Growthinhibition}(\%) = \frac{\text{Growthincontrol} - \text{Growthintreatment}}{\text{Growthincontrol}} \times 100 \qquad \text{(Eq.1)}$$

Quantitative analysis for free radical scavenging capability

Methanolic plant extract of *E. tirucalli* was analyzed using DPPH (1, 1-diphenyl-2-picrylhydrazyl) for its free radical scavenging capacity (Alam et al., 2008). Solution of DPPH 0.05% w/v was made in methanol (95%). The plant extract (0.4 g) was mixed with 10 mL methanol for 4% standard solution. 2 mL of 0.05 mM fresh DPPH solution was added in sterilized test tubes. The stock solution of *E. tirucalli* by serial dilutions (200 µL to 1500 µL) was mixed in each test tube to make it up to 4 mL. After half an hour, the absorbance was recorded using UV – visible spectrophotometer (BMS UV-2600) at 517 nm. The reference standard was methanol, and control was made by adding the same volume of DPPH without plant extract. 95% methanol was served as blank. By the given equation percentage scavenging activity of DPPH was measured:

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$$\% \text{ Radical Scavenging Activity} = \left[\left[\frac{(\text{Absorbance of the control} - \text{Absorbance of the test sample})}{\text{Absorbance of the control}}\right]\right] \times 100 \quad \text{(Eq.2)}$$

The inhibition curve was plotted and depicted as % of mean inhibition ± standard deviation.

Solvent partitioning bioassay

Dried plant material (ground) was drenched in methanol (200 mL) for two days for solvent partitioning bioassay using organic solvents. After evaporation, the methanolic gummy mass (9 g) was apportioned at ambient temperature with *n*-butanol, ethyl acetate, chloroform and *n*-hexane via a separating funnel (Sherazi et al., 2016). These four separated organic fractions have been tested against *C. gloeosporioides in vitro*. Fungal inoculums were made using saline solution, and each organic fraction consumed two concentrations, i.e. 0.5% and 1%. The experiment was done by adding 0.75, and 1.5 mL of all crude organic fractions into 2% MEA medium and 29.25 and 28.5 mL distilled water making final volume upto 30 mL. Thiophenate methyl (Benzimidazole) was used as a positive control. The experiment was replicated thrice, and 5 mm fungal mycelia discs were added in each replicate. The flasks were incubated over seven days at ambient temperature, and the drop in test fungal biomass had been measured using a formula:

$$\text{Growthinhibition}(\%) = \frac{\text{Growthincontrol} - \text{Growthintreatment}}{\text{Growthincontrol}} \times 100 \quad \text{(Eq.3)}$$

GC-MS (gas chromatography-mass spectrometric) investigation

Bioactive phytochemicals of chloroform fraction of *E. tirucalli* methanolic plant extract were identified through GC-MS analysis. Hewlett Packard series 5890 Gas Chromatograph with Mass Selective Indicator 5973N was selected for the examination. The gas chromatography (GC) was interfaced to a mass spectrometer (MS) equipped with an Elite-5MS (95% dimethyl poly saline/5% diphenyl) fused capillary column (30 × 0.25 µm ID × 0.25 µm Df). Electron impact mode at 70 Ev was used for ionization in the GC-MS analysis. The initial oven temperature was 50 °C for 2 min, increased at 20 °C/min to 280 °C, and held for 10 min. The injection was completed in a split-less manner with injection port temperature retained at 250 °C. Data acquisition was accompanied in the MS scan mode (range 40-650 m/z). The comparative percentage area of every constituent was deliberated by comparing it to the entire range (Yusoff et al., 2017).

Statistical analysis

Data obtained in this study were statistically evaluated using variance (ANOVA) analysis to determine variability among groups by Statistix 8.1 software at a significance level of $P \leq 0.05$.

Results

Antifungal activity of methanolic extract of E. tirucalli

All the tested concentrations of methanolic extract of *E. tirucalli* significantly suppressed the *in vitro* growth of *C. gloeosporioides*. However, out of five concentrations, two conc. 2.5% and 3% showed a maximum decline in the test fungal

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biomass, i.e. 64% and 77%. Whereas 46% retardation was observed in 2% concentration as compared to control treatment. The most nominal growth was shown by 1.5% and 1% concentration with 32% and 23% retardation in the test fungus biomass (*Fig. 1*).

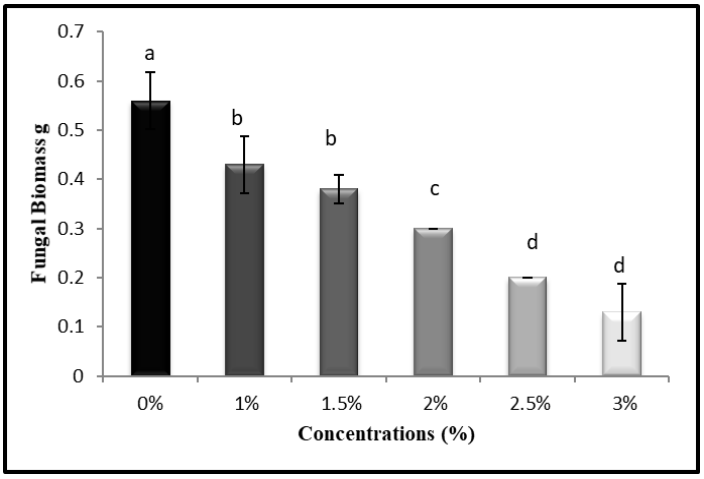

Figure 1. *In vitro results of methanol extract of E. tirucalli against test fungus C. gloeosporioides. The straight bars appear as the standard error of means of three replicates determined by LSD (P = 0.05). Lowercase letters show insignificant variations checked by the LSD test*

Phytochemical analysis

Phytochemical analysis of *E. tirucalli* revealed various bioactive compounds in test plants, i.e. alkaloids, saponins, terpenoids, flavonoids, and tannins. However, glycosides, phlobatannins and coumarins were absent (*Table 1*).

Table 1. *Phytochemical screening of Euphorbia tirucalli*

Groups	Status*
Glycosides	-
Tannins	+
Flavonoids	+
Saponins	+
Terpenoids	+
Alkaloids	+
Phlobatannins	-
Coumarins	-

*(+) means presence (-) means an absence

Antioxidant assay

E. tirucalli antioxidant activity was measured through 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay. The outcomes of this experiment showed that the extract concentrations at all doses significantly exhibited high absorbance than control. At 200 µL concentration of methanol extract revealed 81% radical scavenging activity.

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Figure 2 depicted the scavenging activity of *E. tirucalli* methanolic extract. The action was enhanced by increasing the sample extract concentration. The IC_{50} value for the test sample was recorded as 0.3801 using Graph Pad Prism 8.

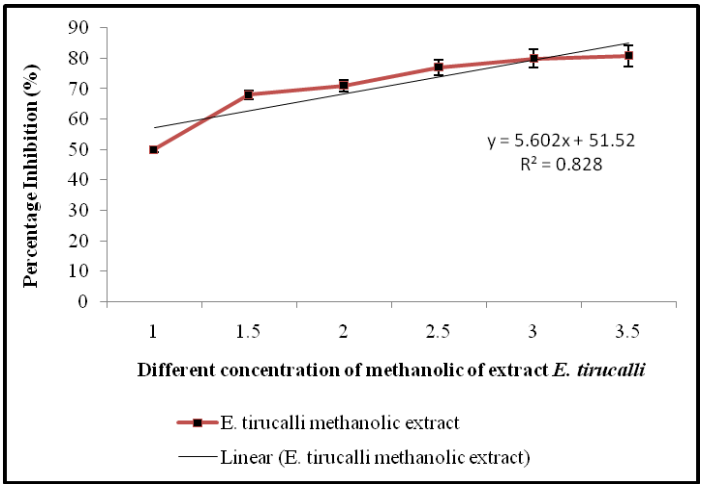

Figure 2. *DPPH radical scavenging activity of the methanol extract of E. tirucalli. Data were represented as % of Mean inhibition ± SD of the experiment at the significance level (P = 0.05)*

Antifungal activity (In-vitro) of extracted organic fractions of E. tirucalli

The test plant methanol extract was apportioned with many organic solvents, namely ethyl acetate, chloroform, *n*-hexane and *n*-butanol. The *in vitro* activities of these fractions were tested. Significant retardation of 90% and 95% was exhibited by two concentrations (0.5% and 1%) of chloroform extract of *E. tirucalli* after seven days of the incubation period (*Fig. 3*). While 0.5% and 1% *n*-hexane concentration caused 80% inhibition in the test fungus biomass, ethyl acetate gave 40% and 50% reduction and the minor fungal growth reduction was recorded in *n*-butanol fraction, i.e. 36% and 54%, respectively. In addition, positive control, i.e. thiophenate methyl (Benzimidazole), was also checked against *C. gloeosporioides*, and inhibition was recorded as 44% and 46%.

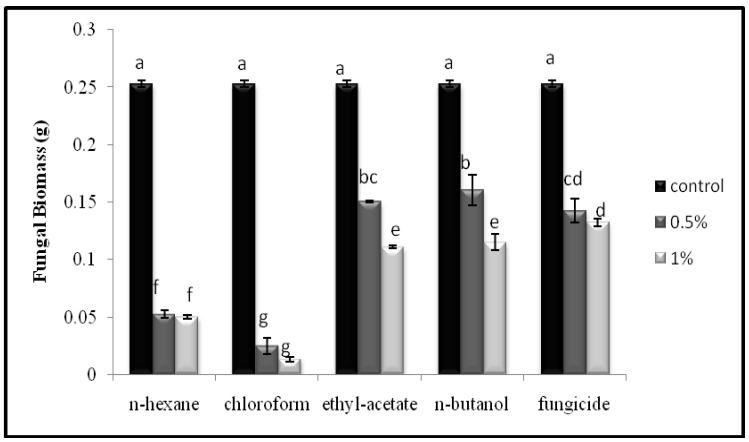

Figure 3. *In vitro results of various organic solvent extracts of E. tirucalli against C. gloeosporioides. The standard error represented by straight lines. Lowercase letters show significant variations checked by LSD (P = 0.05)*

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GC-MS analysis of chloroform extract of E. tirucalli

The present study has identified eleven compounds in the chloroform extract of *E. tirucalli* by GC-MS analysis. The peak report of the complete ion chromatogram has the specifics of peak number, retention time (RT), the name of the compound, molecular formula, molecular weight (MW) and area percentage as presented in *Table 2*. In addition, the disintegration patterns of the peak were characterized and likened to the mass spectra of the constituents with NIST libraries. A chromatogram with the peaks of the test constituents regarding retention time is shown in *Figure 4*. The compounds *n*-hexadecanoic acid with RT 18.676 has peak area 40.446%, 9, 12-octadecadienoic acid (Z, Z) – with RT 20.948 has peak area 22.370%, dodecanoic acid with RT 14.418 has peak area 10.258%, tetradecanoic acid with RT 16.662 with peak area 7.491% and phytol with RT 19.908 has peak area 7.359% were recognized as main antifungal constituents. Most active compounds have an identical name or synonym name, which is essential for searching their function and application.

Table 2. *Phytoconstituents found in the chloroform extract of E. tirucalli L. by GC-MS*

Sr. No	RT (Min)	Constituents	Molecular formula	Molecular weight	Peak area (%)
1	14.418	Dodecanoic acid	$C_{12}H_{24}O_2$	200.317	10.258
2	15.965	10-heneicosene (c,t)	$C_{21}H_{44}$	296.58	1.938
3	16.662	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	7.491
4	17.138	2-pentadecanone,6,10,14-trimethyl-	$C_{18}H_{36}O$	268.5	1.654
5	17.688	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.3975	1.509
6	18.676	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.430	40.446
7	19.159	9,10-dimethyltricyclo [4.2.1.1(2,5)]decane-9,10-diol	$C_{12}H_{20}O_2$	196.29	2.717
8	19.908	Phytol	$C_{20}H_{40}O$	296.539	7.359
9	20.948	9,12-octadecadienoic acid(Z,Z)-	$C_{18}H_{32}O_2$	280.455	22.370
10	24.852	Scopolamine	$C_{17}H_{21}NO_4$	303.353	4.258
11	24.85	Methoxyaceticacid,2-tetradecylester	$C_{17}H_{34}O_3$	286.4	4.258

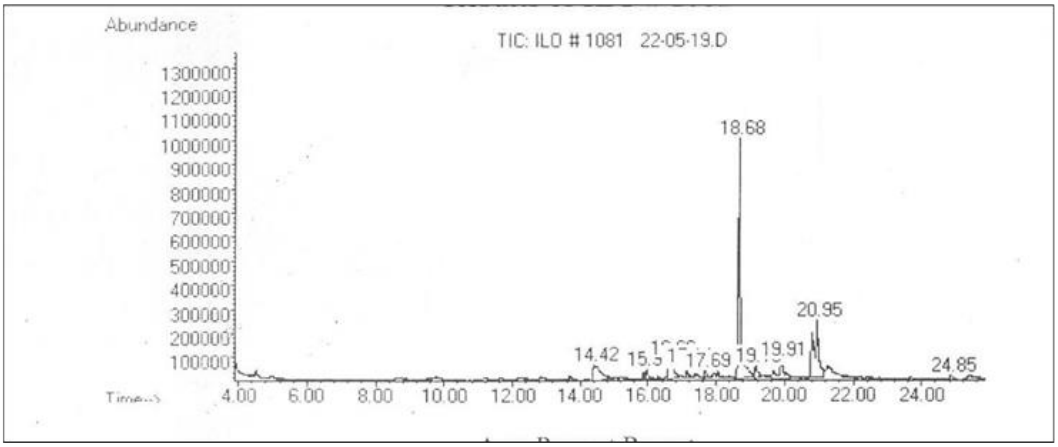

Figure 4. *The chromatogram of GC-MS of E. tirucalli chloroform extract*

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Discussion

Mango is one of the most valuable fruits in tropical and subtropical regions globally, both for domestic and foreign consumption (Khan et al., 2015). Asia dominates the global mango industry with 74.3 per cent of demand, led by America, Africa, and Oceania (Lawson et al., 2019). It is commonly known as "King of the Fruits," produced in around 90 countries around the globe as a reputable evolving tropical trade crop (Alam and Khan, 2001; Tahir et al., 2012). However, mangoes suffer a significant economic loss due to post-harvest decay caused by *C. gloeosporioides* (Konsue et al., 2020). Consumption of synthetic fungicides provides the initial protection for managing post-harvest fungal decay of fruits. To control and lessen chemical preservatives, natural plant sources have been utilized. Many indigenous plant extracts have been identified and reported as antifungal agents against *C. gloeosporioides.* Hence, the present investigation was done to check the antifungal extent of *E. tirucalli* for controlling of anthracnose disease of mango. All the tested concentrations (1-3%) retarded the biomass of the test fungus. Significant reduction in the test fungal biomass was observed in 2.5% and 3% concentration, i.e. 64% and 77%. Other applications of methanolic extract of the test plant also inhibited the fungal mycelium. Previous reports have shown that *in vitro* bioassay of *E. hirta* methanol extract caused a significant decrease in the colony diameter of *C. gloeosporioides* (Khan et al., 2018). Earlier Parekh et al. (2005) also described that methanol and aqueous extracts of *E. hirta* and *E. tirucalli* showed significant antibacterial exploitation. Upadhyay et al. (2010) reported that *E. tirucalli* methanolic leaf extract exhibited intense antifungal activity against *Aspergillus fumigatus, A. flavus* and *Fusarium oxysporum.*

Phytochemical analysis of *E. tirucalli* exhibited various bioactive compounds like alkaloids, terpenoids, saponins, flavonoids, and tannins. Literature supports the current work as *E. tirucalli* contains potential phytochemical constituents that showed antifungal and antimicrobial activities (Jyothiet al., 2008; Avelar et al., 2011; Inbathamizh and Padmini, 2012), but no report is available on their activity against *C. gloeosporioides*. These phytochemicals or bioactive compounds also implicate the antioxidant activity of plants. *E. tirucalli* antioxidant activity was measured through 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay. Outcomes of this experiment showed that the extract concentrations at all doses exhibited high absorbance than control. For example, at 200 µL concentration, methanol extract revealed 81% radical scavenging activity. Previously Jyothi et al. (2008) ascertained that aqueous extract of *E. tirucalli* at 100 µg mL^{-1} concentration showed more significant hydroxyl radical scavenging activity. The test is based on the capacity of a steady free radical, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), to decolourize in antioxidants presence. Furthermore, the radical scavenging activity of DPPH has been revealed to be directly linked with the total phenolic content found in the extract as proposed by various earlier reports (Saeed et al., 2012; Middha et al., 2013).

Methanolic extract of test plant was partitioned with different organic solvents viz. ethyl acetate, chloroform, *n*-hexane and *n*-butanol. Results showed the highest activity up to 90% - 95% by chloroform fraction and fungicide against targeted fungus. Literature researched with*in vitro* appraisal of chloroform, petroleum ether, acetone, hexane and methanol stem extracts of *E. tirucalli* and its fungicidal effects have been established against *Aspergillus niger, A. fumigatus* and *Candida albicans*. The study's outcome showed that the *E. tirucalli* acetone extracts were effective against the test organisms (Prasad et al., 2011).

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GC-MS investigation of the chloroform fraction of *E. tirucalli* was used to identify the bioactive compounds. Over-all eleven compounds were identified (*Table 2*), and among them, the major constituents are *n*-hexadecanoic acid (40.446%), 9, 12-octadecadienoic acid (Z, Z) – (22.370%), dodecanoic acid (10.258%), tetradecanoic acid (7.491%) and phytol (7.359%). *n*-hexadecanoic acid (palmitic acid) recognized in the present study was observed as the largest constituent of fatty acids from Euphorbia species according to Ertas et al. (2015), similar to the results of our study. According to literature, fatty acids exhibit antifungal and antibacterial activity (Dilikaet al., 2000; McGraw et al., 2002). The second major compound found in *E. tirucalli* is 9, 12-octadecadienoic acid (Z, Z) – commonly known as linoleic acid is an unsaturated fatty acid. This compound has been proven to inhibit the development of the phytopathogenic fungi *Crinipellis perniciosa, Pyrenophora avenae, Rhizoctonia solani* and *Pythium ultimum* (Walters et al., 2004). Dodecanoic acid and dodecyclic acid are other names for lauric acid, commonly found in animal and plant oils and fats, especially palm kernel oil and coconut oil identified in current research (Tangwatcharin et al., 2012). Previously, Yenjit et al. (2010) reported that *in vitro* tests of *Areca catechu* L. revealed three triterpenes, and one fatty acid, lauric acid, could inhibit *C. gloeosporioides* mycelial development. Tetradecanoic (myristic acid) is also a common natural fatty acid, and literature search has also proved that palmitic and myristic acids exhibited bioactivity against moulds (Altieri et al., 2009). In addition, 3,7,11,15-tetramethyl-2-hexadecen-1-ol a (phytol) is an acyclic diterpene alcohol. Radulovicet al. (2006) tested phytol against a panel of microorganisms, and this compound proved to exhibit strong antimicrobial properties. Previous studies have shown that all eleven compounds exhibited antifungal activities, as presented in *Table 3*.

Table 3. *List of compounds found in the chloroform extract of E. tirucalli L. and its antifungal activity reported previously*

No	Name of compound/common name	Compound nature	Antifungal activity (references)
1	Dodecanoic acid/Lauric acid	Saturated fatty acid	Inhibit spore germination of *Aspergillus niger* (Rihakova et al., 2001)
2	10-heneicosene (c,t)/Heneicosane	Saturated hydrocarbon	Exhibit antifungal activity on the growth *Penicilliumsp, C. albicans, A. glaucus* and *T. viride* (Mihailovicet al., 2011)
3	Tetradecanoic acid/Myristic acid	Saturated fatty acid	Exert bioactivity against moulds (Altieri et al., 2009)
4	2-pentadecanone,6,10,14-trimethyl-/Phytone	Sesquiterpenoids	Exhibit antibacterial and antifungal properties (Yoo et al., 2008; Seidel et al., 2004)
5	Pentadecanoic acid	Saturated fatty acid	Antibacterial and antifungal activity of *Excoecaria agallocha* (Agoramoorthyet al., 2007)
6	n-hexadecanoic acid/Palmitic acid	Saturated fatty acid	Antifungal activity of palmitic acid against *Candida spp* (Belarmino de Souza et al., 2015)
7	9,10-dimethyltricyclo [4.2.1.1(2,5)]decane-9,10-diol	Alcohol	Showed antifungal activity against *Macrophomoina phaseolina* (Javaid et al., 2017)
8	3,7,11,15-tetramethyl-2-hexadecen-1-ol/Phytol	Diterpene alcohol	Antimicrobial activity of *Equisetum arvense* L. against *Aspergillus niger* and *Candida albicans* (Radulovicet al., 2006)

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9	9,12-octadecadienoic acid(Z,Z)-/Linoleic acid	Unsaturated fatty acid	Exhibit antifungal activity against *Crinipellis perniciosa, Rhizoctonia solani, Pyrenophora avenae* and *Pythium ultimum* (Walters et al., 2004)
10	Hyoscine/Scopolamine	Alkaloid	The fungistatic activity of hyoscyamine and scopolamine against *Magnaporthe oryzae* and *Rhizoctonia solani* (Abdel-Motaalet al.,2010)
11	Methoxyaceticacid,2-tetradecylester	Fatty acid	Efficacy of Volatile organic compounds in suppression of Verticillium wilt of cotton (Zhang et al.,2015)

Conclusion

This study has demonstrated that all compounds identified from *Euphorbia tirucalli* have exhibited significant antifungal potential against the target fungus. Thus, further investigations of the active compounds present in the test plant are essential to establish *E. tirucalli* as an antifungal plant against *C. gloeosporioides*. The plant extract also showed significant antioxidant properties, indicative of its potential as a source. *E. tirucalli* can be used to make a variety of modern bio fungicides that can be used to combat various phytopathogens, including mango anthracnose.

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