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

Khan, B.1 – Jabeen, K.1* – Kanwal, Q.2 – Iqbal, S.1

1Department of Botany, Lahore College for Women University, Lahore, Punjab 54000, Pakistan
2Department of Chemistry, University of Lahore, Raiwind Road Campus, Lahore, Punjab, Pakistan

*Corresponding author
e-mail: Khajista_1@hotmail.com; phone: +92-334-993-1766

(Received 25th Dec 2020; accepted 14th May 2021)

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 gloeosporioides 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 (Kengaantalet 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).
Various fungicides, such as copper hydroxide, azoxystrobin, mancozeb, prohloraz and copper sulfate products, are used to treat infection of *C. gloeosporioides* (Dirouet et al., 2005; Sundravadana et 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 antimicrobial 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*. 
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) DH2O 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:

\[
\text{Growth Inhibition} (\%) = \frac{\text{Growth}_{\text{control}} - \text{Growth}_{\text{treatment}}}{\text{Growth}_{\text{control}}} \times 100 \quad (\text{Eq.1})
\]

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:
% Radical Scavenging Activity = \left[ \frac{\text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \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:

\[
\text{Growth Inhibition} (\%) = \frac{\text{Growth in control} - \text{Growth in test}}{\text{Growth in control}} \times 100 \quad \text{(Eq.3)}
\]

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

![Graph showing DPPH radical scavenging activity](image)

**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%.

![Graph showing in vitro results of various organic solvent extracts](image)

**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)
**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_{30}O_{2} | 228.37           | 7.491         |
| 4     | 17.138   | 2-pentadecanone,6,10,14-trimethyl- | C_{15}H_{30}O_{2} | 268.5            | 1.654         |
| 5     | 17.688   | Pentadecanoic acid | C_{15}H_{30}O_{2} | 268.5            | 1.654         |
| 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*
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 (Jyothi et 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 within 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).
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 (Dilika et 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 (phytol) is an acyclic diterpene alcohol. Radulovic et 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 of *Penicillium* spp, *C. albicans*, *A. glaucus* and *T. viride* (Mihailovic et 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 | | |
| 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 *Macrophomina 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* (Radulovic et al., 2006) |
|   | Compound Description                                      | Property                          | Reference                                                                 |
|---|------------------------------------------------------------|-----------------------------------|----------------------------------------------------------------------------|
| 9 | 9,12-octadecadienoic acid (Z,Z)-/Linoleic acid             | Unsaturated fatty acid            | Exhibit antifungal activity against Crinipellis perniciosa, Rhizoctonia solani, Pyrenophora avenue 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-Motaal et al., 2010) |
| 11| Methoxyacetic acid, 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 biofungicides that can be used to combat various phytopathogens, including mango anthracnose.

**Acknowledgements.** The authors are thankful to Botany Department, Lahore College for Women University, Lahore, Pakistan, and its support in this study is acknowledged.

**REFERENCES**

[1] Abad, M. J., Ansuategui, M., Bermejo, P. (2007): Active antifungal substances from natural sources. – Archive for Organic Chemistry 7: 116-145.

[2] Abdel-Motaal, F. F., El-Zayat, S. A., Kosaka, Y., El-Sayed, M. A., Kashima, R., Maeda, Y., Nassar, M. S. M. (2010): Antifungal activities of hyoscyamine and scopolamine against two major rice pathogens: Magnaporthe oryzae and Rhizoctonia solani. – Journal of General Plant Pathology 76(76): 102-111.

[3] Agoramooorthy, G., Chandrasekaran, M., Venkatesalu., Hsu, M. J. (2007): Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. – Brazilian Journal of Microbiology 38(4).

[4] Alam, S. M., Khan, M. A. (2001): Mango - an important fruit of Pakistan. – Industry and Economy.

[5] Alam, M., Ghani, A., Subhan, N., Rahman, M., Haque, M., Majumder, M. (2008): Antioxidant and membrane stabilizing properties of the flowering tops of *Anthocephalus cadamba*. – Natural Product Communications 3(65): 7.

[6] Altieri, C., Bevilacqua, A., Cardillo, D., Sinigaglia, M. (2009): Antifungal activity of fatty acids and their monoglycerides against *Fusarium spp* in a laboratory medium. – International Journal of Food Science and Technology 44(2): 242-245.

[7] Araujo, K. M., Lima, A. D., Silva, J. N., Rodrigues, L. L., Amorim, A. G. N., Quelemes, P. V., Santos, R. C., Rocha, J. A., Andrades, E. O., Leite, J. S. A., Mancini-Filho, J., Trindade, R. A. (2014): Identification of phenolic compounds and evaluation of antioxidant and antimicrobial properties of *Euphorbia tirucalli* L. – Antioxidants 3: 159-175.
[8] Avelar, B. A., Lélis, F. J. N., Avelar, R. S., Weber, M., Souza-Fagundes, E. M., Lopes, M. T. P. (2011): The crude latex of Euphorbia tirucalli modulates the cytokine response of leukocytes, especially CD4+ T lymphocytes. – Revista Brasileira de Farmacognosia 21(4): 662-667.

[9] Bashir, S., Jabeen, K., Iqbal, S., Javed, S., Naem, A. (2019): Lantana camara: phytochemical analysis and antifungal prospective. – Plantadaninha 37: 1-7.

[10] Belarmino de Souza, T., Bretas, A. C. O., Alves, R. J., Magalhaes, T. F. F., Stoianoff, M. A. R. (2015): Synthesis and antifungal activity of Palmitic acid-based neoglycolipids related to Papulacandin D. – Artigo 38(10): 1282-1288.

[11] Dean, R., Van, Kan, J. A., Pretorious, Z. A., Hammond-Kosack, K. E., Pietro, A. D. I., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., Foster, G. D. (2012): The Top 10 fungal pathogens in molecular plant pathology. – Molecular Plant Pathology 13: 414-430.

[12] Dessalegn, Y., Ayalew, A., Woldetsadik, K. (2013): Integrating plant defense inducing chemical, inorganic salt and hot water treatments for the management of post-harvest mango anthracnose. – Post-harvest Biology and Technology 85: 83-88.

[13] Dilika, F., Brenner, P. D., Meyer, J. J. M. (2000): Antibacterial activity of linoleic and oleic acids isolated from Helichrysum pedunculatum: a plant used during circumcision rites. – Fitoterapia 71: 450-452.

[14] Dirou, J., Stovold, G. (2005): Fungicide management program to control mango anthracnose. – Prime Fact 19.

[15] Dong, J., Zhang, L., Liu, Y., Xu, N., Zhou, S., Yang, Q., Yang, Y., Ai, X. (2020): Thymol protects channel catfish from Aeromonas hydrophila Infection by inhibiting aerolysin expression and biofilm formation. – Microorganisms 8: 636.

[16] Dubey, N., Srivastava, B., Kumar, A. (2008): Current status of plant products as botanical pesticides in storage pest management. – Journal of Biopesticide 1(2): 182-186.

[17] Ertas, A., Yılmaz, M. A., Firat, M. (2015): Chemical profile by LC-MS/MS, GC/MS and antioxidant activities of the essential oils and crude extracts of two Euphorbia species. – Natural Product Research 29: 529-534.

[18] Farr, D. F., Aime, M. C., Rossman, A. Y., Palm, M. E. (2006): Species of Colletotrichum on agavaceous. – Mycological Research 110: 1395-1408.

[19] Gulcin, I., Uguz, M. T., Oktay, M., Beydemir, S., Kufrevioglu, O. I. (2004): Evaluation of the antioxidant and antimicrobial activities of Clary Sage (Slavia scclarea L.). – Turkish Journal of Agriculture and Forestry 28: 25-33.

[20] Gupta, N., Vishnoi, G., Wal, A., Wal, P. (2012): Medicinal value of Euphorbia tirucalli. – Systematic Reviews in Pharmacy 4(1): 40.

[21] Hanif, S., Jabeen, K., Iqbal, S. (2017): Management of damping off disease by extracts of Albizia lebbeck (L.) Benth. – Bangladesh Journal of Botany 46(3): 1009-1014.

[22] Huang, C. Y., Kuo, C. H., Wu, C. H., Kuan, A. W., Guo, H. R., Lin, Y. H., Wang, P. K. (2018): Free radical scavenging, anti-inflammatory, and antibacterial activities of water and ethanol extracts prepared from compressional-puffing pretreated mango (Mangifera indica L.) peels. – Journal of Food Quality 1025387.

[23] Inbathamizh, L., Padmini, E. (2012): Gas chromatography-mass spectrometry analyses of methanol extract of Moringa oleifera flowers. – International Journal of AnalyticalBio-Science 3(5): 1394-1397.

[24] Jahan, N., Khalil-Ur-Rehman., Ali, S., Bhatti, I. A. (2011): Antimicrobial potential of gemmo-modified extracts of Terminalia arjuna and Euphorbia tirucalli. – International Journal Agriculture and Biology 13(6): 1001-1005.

[25] Javeid, A., Quds, H., Shoaiab, A. (2017): Bioassay guided fractionation of Senna occidentalis for identification of natural antifungal constituents against Macrophomina phaseolina. – Planta Daninha 35. https://doi.org/10.1590/S0100-83582017350100002.
[26] Jyothi, T. M., Shankariah, M. M., Prabhu, K., Lakshminarasu, S., Srinivasa, G. M., Ramachandra, S. S. (2008): Hepatoprotective and antioxidant activity of Euphorbia tirucalli. – Iranian Journal of Pharmacology and Therapeutics 7(1): 25-30.

[27] Kenganal, M., Ranebennur, H., Byadgi, A. S. (2010): Management of anthracnose in mango. – University of Agricultural Sciences, Dharwad Karnataka. Online Edition of India's National Newspaper.

[28] Khan, A. S., Ali, S., Khan, I. A. (2015): Morphological and molecular characterization and evaluation of mango germplasm: an overview. – Scientia Horticulturae 194: 353-366.

[29] Khan, B., Jabeen, K., Iqbal, S. (2018): Fungistic potential of Euphorbia hirta L. against the cause of anthracnose. – Romanian Biotechnological Letters 23(6): 1-10.

[30] Konsue, W., Dethoup, T., Limtong, S. (2020): Biological control of fruit rot and anthracnose of post-harvest mango by antagonistic yeasts from economic crops leaves. – Microorganisms 8: 317.

[31] Kumar, A. S., Reddy, N. P. E., Reddy, K. H., Devi, M. C. (2007): Evaluation of fungicidal resistance among Colletotrichum gloeosporioides isolates causing mango anthracnose in agri export zone of Andhra Pradesh, India. – Plant Pathology Bulletin 16: 157-160.

[32] Lawson, T., Grantley, W. L., Asgar, A., Chiew, F. C. (2019): Characterization of Southeast Asia mangoes (Mangifera indica L) according to their physicochemical attributes. – Scientia Horticulturae 243: 189-196.

[33] Li, R. W., Leach, D. N., Myers, P., Leach, G. J., Lin, G. D., Brushett, D. J., Waterman, P. G. (2004): Anti-inflammatory activity, cytotoxicity and active compounds of Tinospora smilacina Benth. – Phytotherapy Research 18: 78-83.

[34] Mcgraw, L. J., Jager, A. K., Van Staden, J. (2002): Isolation of antibacterial fatty acids from Schotia brachypetala. – Fitoterapia 73: 431-433.

[35] Memon, N. A. (2016): Mango: Pakistan 4th largest Producer in the World. – Pakistan Food Journal 24-26.

[36] Middha, S. K., Ushat., Pande, V. (2013): HPLC evaluation of phenolic profile, nutritive content, and antioxidant capacity of extracts obtained from Punica granatum fruit peel. – Advances in Pharmacological Sciences. https://doi.org/10.1155/2013/296236.

[37] Mihailovic, V., Vukovic, N., Niciforovic, N., Solujic, S., Mladenovic, M., Maskovic, P., Stankovic, M. S. (2011): Studies on the antimicrobial activity and chemical composition of the essential oils and alcoholic extracts of Gentiana asclepiadea. – Journal of Medicinal Plants Research 5(7): 1164-1174.

[38] Munawwar, M., Jabeen, K., Iqbal, S. (2018): Biochar and compost antifungal activity against Botrytis Cinerea Pers. Ex. Fe. – Bangladesh Journal of Botany 47(1): 141-146.

[39] Mwine, J., Damme, P. V. (2010): Evaluation of selected pesticidal plant extracts against major cabbage insect pests in the field. – Tropentag: "World Food System - A Contribution from Europe," ETH Zurich, Switzerland.

[40] Pandey, A., Yadava, L. P., Manoharan, M., Chauhan, U. K., Pandey, B. K. (2012): Effectiveness of cultural parameters on the Growth and sporulation of Colletotrichum gloeosporioides causing anthracnose disease of Mango (Mangifera indica L.). – Online Journal of Biological Sciences 12: 123-133.

[41] Parekh, J., Jadeja, S., Chanda, S. (2005): Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. – Turkish Journal of Biology 29: 203-210.

[42] Perea, S., Patterson, T. F. (2002): Antifungal resistance in pathogenic fungi. – Clinical Infectious Diseases 35: 1073-1080.

[43] Phoulivong, S., Cai, L., Chen, H., McKenzie, E. H. C., Abdelsalam, K., Chukeatirote, E., Hyde, K. D. (2010): Colletotrichum gloeosporioides is not a common pathogen on tropical fruits. – Fungal Divers. 44: 33-43.
[44] Prasad, S., Swapna, N. L., Prasad, M. (2011): Efficacy of Euphorbia tirucalli (L) towards microbicidal activity against human pathogens. – International Journal of Pharma and Bio Sciences 2: 12-18.

[45] Pscheidt, J. W., Ocamb., C. M. (2018): Pacific Northwest Plant Disease Management Handbook [online]. – Oregon State University, Corvallis, OR.

[46] Radulovic, N., Stojanovic, G., Palic, R. (2006): Composition and antimicrobial activity of Equisetum arvense L. essential oil. – Phytotherapy Research 20(1): 85-88.

[47] Rihakova, Z., Plockova, M., Filip, V., Smidral, J. (2001): Antifungal activity of lauric acid derivatives against Aspergillus niger. – European Food Research and Technology 213(6): 488-490.

[48] Saeed, N., Khan, M. R., Shabbir, M. (2012): Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. – BMC Complementary and Alternative Medicine 12: 221.

[49] Saaco, V. G. (2017): Trends in world mango production and marketing. – Acta Horticulture 1183: 351-363.

[50] Schulze-Kayser, N., Feuerisen, M. M., Schieber, A. (2015): Phenolic compounds in edible species of the Anacardiaceae family - a review. – RSC Advances 5: 73301-73314.

[51] Seidel, V., Taylor, P. W. (2004): In-vitro activity of extracts and constituents of Pelargonium against rapidly growing mycobacteria. – International Journal of Antimicrobial Agents 23: 613-619.

[52] Shah, K. A., Patel, M. B., Patel, R. J., Parmar, P. K. (2019): Mangifera indica (Mango). – Pharmacognosy Reviews 4: 42-48.

[53] Sherazi, A. Z., Jabeen, K., Iqbal, S., Yousaf, Z. (2016): Management of Ascochyta rabiei by Chenopodium album extracts. – Planta Daninha 34(4): 675-680.

[54] Silva, P. R., Rabelo, R. A. S., Terra, A. P. S., Teixeira, D. N. S. (2008): Susceptibility to antifungal agents among Cryptococcus neoformans varieties isolated from patients at a university hospital. – Revista da Sociedade Brasileira de Medicina Tropical 41: 158-162.

[55] Slavin, J. L., Lloyd, B. (2012): Health benefits of fruits and vegetables. – Advances in Nutrition 3: 506-516.

[56] Sundravadana, S., Alice, D., Kuttalam, S., Samiyappan, R. (2006): Control of mango anthracnose by Azoxystrobin. – Tunisian Journal of Plant Protection 1: 109-114.

[57] Tahir, W. A., Ahmad, M., Iftikhar, M. (2012): An analysis of the effectiveness of extension work conducted by public sector with special reference to mango in the southern Punjab, Pakistan. – Pakistan Journal of Agricultural Sciences 49: 229-232.

[58] Tangwatcharin, P., Khopaibool, P. (2012): Activity of virgin coconut oil, lauric acid or monolaurin in combination with lactic acid against Staphylococcus aureus. – Southeast Asian Journal of Tropical Medicine and Public Health 43(4): 969-985.

[59] Tiwari, S., Singh, A. (2006): Biochemical stress response in freshwater fish Channa punctatusinduced by aqueous extracts of Euphorbia tirucalli plant. – Chemosphere 64: 36-42.

[60] Topa, S. H., Palombo, E. A., Kingshott, P., Blackall, L. L. (2020): Activity of cinnamaldehyde on quorum sensing and biofilm susceptibility to antibiotics in Pseudomonas aeruginosa. – Microorganisms 8: 455.

[61] Upadhyay, B., Singh, K. P., Kumar, A. (2010): Ethno-medicinal, phytochemical and antimicrobial studies of Euphorbia tirucalli L. – Journal of Phytophylology 2(4): 65-77.

[62] Walters, D., Raynor, L., Mitchell, A., Walker, R., Walker, K. (2004): Antifungal activity of four fatty acids against plant pathogenic fungi. – Mycopathologia 157: 87-90.

[63] Wang, S., Melnyk, J. P., Tsao, R., Marcone, M. F. (2011): How natural dietary antioxidant in fruits, vegetables and legumes promote a vascular health. – Food Research International 44: 14-22.

[64] Wei, Y., Pu, J., Zhang, H., Liu, Y., Zhou, F., Zhang, K., Liu, X. (2017): The laccase gene (LAC1) is essential for Colletotrichum gloeosporioides development and virulence on...
mango leaves and fruits. – Physiological and Molecular Plant Pathology. DOI: 10.1016/j.pmpp.2017.03.005.

[65] Wootten-Beard, P. C., Ryan, L. (2011): Improving public health. The role of antioxidant-rich fruit and vegetables beverages. – Food Research International 44: 3135-3148.

[66] Yenjit, P., Issavakraisila, M., Intana, W., Chantrapromma, K. (2010): Fungicidal activity of compounds extracted from the pericarp of Areca catechu against Colletotrichum gloeosporioides in vitro and in mango fruit – Postharvest Biology and Technology 55(2): 129-132.

[67] Yoo, Y. M., Nam, J. H., Kim, M. Y., Choi, J., Parz, H. J. (2008): Pectolinarin and pectolianarigenin of Cirsium setidens prevent the hepatic injury in rats caused by D-galactosamine via an antioxidant mechanism. – Biological and Pharmaceutical Bulletin 31: 760-764.

[68] Yusoff, E., Ahmad, A., Mohamad, S., Muhammad, N. F. (2017): GC-MS analysis of some volatile constituents extracted from stem of Euphorbia tirucalli Linn. – Archives of Orofacial Sciences 12(1): 36-44.

[69] Zhang, Q., Yang, L., Zhang, J., Wu, M., Chen, W., Jiang, D., Li, G. (2015): Production of antifungal volatiles by non-pathogenic Fusarium oxysporum and its efficacy in suppression of Verticillium wilt of cotton. – Plant and Soil 392(1-2): 101-114.