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Research Article

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# GC-MS profiling and antifungal activities of *Morinda citrifolia* L. leaf extract against fungal pathogens of crown rot disease of banana

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## ABSTRACT

The increasing demand for organic agricultural products especially among the elite stimulated the search for safe and alternative means of crown rot disease control. *Morinda citrifolia* is one of the listed medicinal plants among the Polynesian countries. This study documented the phytochemical profile of *M. citrifolia* using GC-MS and their antifungal activities against crown rot pathogens. The key phytochemical constituents of the extract were Phytol 2-Hexadecen-1-ol, (Diterpene) (25.96%), Squalene (Triterpene) (15.13%), 1, 3-Propanediol (Polyphenol) (4.68%), Pyran-4-one 4H-, 9 (Flavonoid), and 2H-1-Benzopyran-6-ol (Vitamins) (5.14%), 2-Cyclohexan-1-one, (Phenol) (2.54%). Fungal pathogens; *Lasiodiplodia theobromae*, *Colletotrichum musae*, *Colletotrichum asianum* and *Fussarium Longipes* isolated from crown rot infected banana fruits during earlier studies were used in this experiment. The poisoned food technique method was adopted in evaluating the inhibitory effect of the leaf extract against the fungal isolates. Results indicated significantly high fungal growth inhibition ($P < 0.05$) in concentration dependent manner on amended PDA media. 100% pathogen radial growth inhibition in all isolates was recorded at 100 mg/mL concentration. Therefore, findings of this study suggest application of leaf extract of *M. citrifolia* is a potential safe and alternative control of banana crown rot.

**KEYWORDS:** Antimicrobial, Fungal pathogens leaf extract, *Morinda citrifolia*, Phytochemicals

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## INTRODUCTION

*Morinda citrifolia* is a tree native to South-East Asia and the most commonly found variety in Malaysia is the var. *citrifolia*. *M. citrifolia* has been cultivated extensively in Malaysia and many tropical regions such as and South America, due to its economic value and health benefits (Srinivasahan & Durairaj, 2014). The fruit, juice, seed, leaf, and root have been used as sources of traditional medicines in different countries in the world to cure many diseases and ailments. Commercially, its products such as fruit juice and capsulated fruit powder extract have gained popularity in Asia, Europe and America (Nelson, 2003). All plant parts exhibited antioxidant, antimicrobial, anti-cancer and anti-inflammatory properties (Assi *et al.*, 2017). According to the findings of McClatchey (2002), *M. citrifolia* is used in the treatment of approximately 2000 ailments around the world. Jayaraman *et al.* (2008), reported all parts of the plant possess antifungal, antibacterial, tumour suppression effects. Zin *et al.* (2007) also reported the ant-oxidant properties of *M. citrifolia* root extract. Similarly, Masuda *et al.* (2009) documented the inhibitory effects of *M. citrifolia* seeds on elastase and tyrosinase enzymes. Findings of Usha *et al.* (2010), reported *M. citrifolia* leaves are used in the treatment of minor infections and ulcerations on the skin.

Similarly, medicinal plants contain a large number of phytochemicals with antimicrobial properties which may serve as good and safe alternative biopesticides due to their low toxicity to humans and the environment (Madhumitha *et al.*, 2012; Adefuye & Ndip, 2013). These phytochemicals are considered alternative sources of broad spectrum biopesticides derived from natural products for the management of plant diseases, due to their varied and complex mechanisms of action against pathogenic organisms (Gurjar *et al.*, 2012; Idris *et al.*, 2015).

The use of Gas Chromatography-Mass Spectrometry (GC-MS) for the identification and quantification of phytochemicals has been on the increase since the technique proved to be a valuable method for the identification of volatile compounds, non-polar components, lipids and fatty acids (Ganesh & Mohankumar, 2017). The GC interfaced with MS is considered a powerful tool

for the detection and analysis of organic compounds (Khan *et al.*, 2017). According to Su *et al.* (2005), bioactive compounds such as polyphenols, alkaloids, glycosides, polysaccharides, lignans, iridoids, morindin, anthroquinones, trisaccharide fatty acid esters, scopoletin, minerals and vitamins have been isolated from *M. citrifolia* fruits, leaves and roots.

Little work has been reported on GC-MS analysis of methanol extract of *M. citrifolia* leaves. Therefore, the present study focused on extraction, GC-MS technique analysis of bioactive components and assessment of fungicidal activities of methanol extract of *M. citrifolia* leaves using the poisoned food technique method.

## MATERIALS AND METHODS

### Collection of Plant Materials

Fully matured leaves of *M. citrifolia* were collected from Taman Pertanian Universiti, Universiti Putra Malaysia (UPM), Selangor, Malaysia. The plant was identified and authenticated by a botanist at the Biodiversity Unit, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), collected leaves were deposited in the Phytomedicinal Herbarium of Biodiversity Unit, IBS, Selangor with a specimen voucher No. SK 3255/17.

### Preparation and Extraction of Plant Materials

Collected leaves were immediately brought to Biological Control Laboratory, at the Department of Plant Protection, Faculty of Agriculture, UPM. The leaves were washed under running tap water to get rid of dust and debris, then rinsed three times with sterile distilled water, then air dried in the laminar flow for 6 hours, then dried in an oven (Memmert, Germany) at 45 °C for 3 days. Five hundred grams of dried leaves were ground in a grinder (Retsch SK100) for 2 min to produce a powder of a uniform size (Rivera *et al.*, 2012). Fifty grams of the powder was dissolved in 500 mL of methanol. The mixture was thoroughly agitated on orbital shaker at 150 rpm for 24 h. The mixture was filtered in three stages; first with double layered muslin cloth, second, the extract was filtered using Whatman No. 1 filter paper and thirdly using a micro (0.45 µm) syringe filter (Bhutia *et al.*, 2016; Kurwadkar *et al.*, 2017). Thereafter leaf filtrate was concentrated under reduced pressure of 40-45 °C using a rotary evaporator (BUCHI R-215, Switzerland) to obtain a viscous semi solid mass. Then, the semi solid mass was transferred to a beaker covered with aluminium foil and dried to powder form in an oven for 6 days at 40-45 °C.

### GC-MS Analysis of *M. Citrifolia* Leaf Extract

GC-MS analysis was performed at the Chemistry Department, Faculty of Science, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia. The analysis was performed to determine the volatile bioactive compounds present in the methanolic extract of *M. citrifolia* and their relative abundance following the methods described by Seotardjo *et al.* (2007) and Khan *et al.* (2017). Two hundred mg of the dried extract powder was dissolved in 2 mL of methanol in a vial. GC-MS analysis of *M. citrifolia* methanol leaves extract was carried out using gas chromatography coupled with a mass spectrometer (GC-MS QP-2010, Shimadzu, Japan) equipped with Zebron ZB5-MS capillary column (30 meters x 0.25 mm I.D. x 0.25µm film thickness) (Jegajeevanram *et al.*, 2014). The capillary was set to an initial temperature of 50 °C, and maintained at this temperature for 3 min. The oven temperature was increased up to 300 °C at the end of the period and the rate of an increase of 10 °C/min and maintained for 10 min. The injection port temperature was set at 250 °C and Helium flow rate at 1.0 mL/min. The ionization voltage was set at 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 35-450 (m/z). The ion source temperature was maintained at 240 °C and the interface temperature was at 300 °C. The MS start time was 3 min, and the end time was 34 min with the solvent cut time was of 2 min and 30 s. Mass spectra were taken over *m/z* range 35-450 atomic mass unit amm. National Institute Standard and Technology (NIST) Ver. 02 MS data library was used for, comparing spectral data of the leaves sample. The mass spectrum of components obtained from GC-MS analysis, compounds' names, molecular weight and structure of the components of the test materials were also confirmed (El-Beltagi *et al.*, 2018).

### Source of Inoculum

Crown rot inciting fungi whose pathogenicity was already established in the department of Plant Protection University Putra Malaysia were isolated from naturally crown rot infected banana fruits sampled from Negeri Sembilan, Melaka and Selangor in Malaysia during the earlier study were used. Isolates were identified morphologically and confirmed molecularly using their rDNA following the method described by Karmakar *et al.* (2016). Generated ITS sequences were accessioned by GenBank as; *Colletotrichum musae* (MG386643.1), *Colletotrichum asianum* (MG386644.1), *Lasiodiplodia theobramae* (MG386642.1), and *Fusarium longipes* (MG386645.1).

### Evaluation of Antifungal Properties of *M. Citrifolia* Leaf Extract

Assessment of the antifungal properties of *M. citrifolia* leaf extract was performed using the poisoned food technique method (Nweke, 2015). To achieve this, 100 mg of powered extract was dissolved in 1 mL of 50% Dimethyl sulfoxide DMSO to produces 100% stock solution. Then, serial dilutions of 50, 60, 70, 80, 90 and 100 mg/mL were prepared by adding 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mL of the 100% stock solution to already prepared 20 mL PDA on Petri dishes (treatments) then allow to solidify as described by Gayathri and Ramesh (2013). Using a sterilized cork borer, 5 mm plug of each purified colony mycelial disc were cut and placed on the centre of the petri dishes containing impregnated PDA, sealed and stored at room temperature of 25 °C ± 2, until the control plates were full. Controls were plates impregnated with 50% DMSO, all treatments were in triplicates (Idris *et al.*, 2015). Antifungal activities of extract were assessed by measuring diameter of

mycelial growth in each treatment, and also by taking the percentage inhibition of radial growth (% PRIG) after 72 hrs and calculated as:

$$\% \text{ PIRG} = \frac{R_1 - R_2}{R_1} \times 100$$

Where: % PIRG = percentage inhibition of radial growth
$R_1$ = radius of fungi colony in control plate
$R_2$ = radius of fungi colony in treated plate

### Scanning Electron Microscopic (SEM) of the Antifungal Activities of *M. Citrifolia* Leaf Extract Against *C. Musae* on Different Concentrations of Extract Amended PDA Media.

SEM microscopy of the antifungal activities of *M. citrifolia* leaf extract was performed on 7 days old *C. musae* growing on four different concentrations of PDA amended media; 50, 60, 70, and 80 mg/mL of leaf extracts and a control following the method described by (Kim *et al*., 2017). From each treatment, four pieces of 1 $cm^3$ fungal mycelial mat were cut and fixed in vials containing 5 mL of 25 mg/mL glutaraldehyde buffer, stored at 4 °C for 24 hours. Specimen were centrifuged at 1500 rpm, then supernatant discarded. Specimen were washed in 0.1 M sodium cacodylate buffer for 10 minutes, 3 times. Samples were further post-fixed in 1% osmium tetroxide at 4 °C for 2 hrs. Then washed 3 times in 0.1 M sodium cacodylate buffer for 10 minutes was repeated. This followed by dehydration in 35%, 50%, 75%, and 95% for 10 minutes in graded acetone series and in 100% acetone for 15 minutes. Dehydrated specimens were placed on albumin coated with aluminium foil prior to critical point drying. Thereafter, samples were transferred onto specimen baskets and kept in a critical-point drier for half an hour, then mounted onto stub by the use of double-sided tape, and gold-coated in ion sputter-coater. Specimen were then observed and photographed on a SEM (BAL-TEC, Model SCD 005 JEOL (InTouchScope, USA).

### Statistical Data Analysis

Data on percentage pathogen growth inhibition was analysed using one-way ANOVA and significant means were separated by Tukey at $P < 0.05$ level of significance.

## RESULTS

### GC-MS Analysis of Methanol Extract of *M. Citrifolia* Leaf

The GC-MS chromatogram of *M. citrifolia* leaf extract showed a total of 85 peaks indicating the presence of several phytochemical constituents. From the numerous peaks, 17 key and principal bioactive components were identified alongside their earlier documented biological activities by previous researchers (Table 1). The identified major phytochemical components were clearly distinguished by their respective peaks areas as shown on the chromatogram. These principal compounds were; Phytol 2-Hexadecen-1-ol (3, 7, 11, 15-tetramethyl), 1,3-Propanediol, (Isobutylglycerol, nitro), 2H-1-Benzopyran-6-ol (Vitamin E), (Pyran-4-one) 4H), 5-Dihydro-6-methyl-3, 2-Cyclohexan-1-one (3-oxo-1-butenyl), Linolenic acid, (alpha-Linolenic acid), 2-Cyclohexan-1-one (3-oxo-1-butenyl), Hexadecanoic acid (Palmitic acid), Tetrahydro-3-furamethanol (Tetrahydro-3-furanylmethanol), Gama-Sitosterol (Stigmast-5-en-3-ol), Stigmasterol (Stigmasta-5,22-dien-3-ol), Larixic acid (Larixinic acid), 3-Deoxy-d-mannanic acid, Cyclohexanemethanol (3-methyl-1-butenyl), Ergost-5-en-3-ol (Ergost-5-en-3beta-ol).

The principal phytochemical components of *M. citrifolia* leaf extract with their percentage peak heights were; Phytol 2- Hexadecen-1-ol (3,7,11,15-tetramethyl) (25.96%), 2,6,10,14,18,22-Tetracosahexaene (15.13%), (All-trans-Squalene) 2H-1-Benzopyran-6-ol (5.14%), 1,3-Propanediol, Isobutylglycerol, nitro (4.68%), alpha-linolenic acid (fatty acid) (4.20%) and Dihydro-6-methyl-3, (Pyran-4-one,) 4H- (3.83%). Similarly, the names and structures of the identified principal phytochemicals compounds that constitutes 50.91% of the total volatile bioactive constituents of *M. citrifolia* using GC-MS technique are shown in Figure 1.

### Assessment of Antifungal Activities of *M. Citrifolia* Leaf Extract

The antifungal properties of 50, 60, 70, 80, 90 and 100 mg/mL of *M. citrifolia* leaf extract against mycelial growth of fungal pathogens were studied. Results given in Tables 2 and 3, showed a highly significant effect of extract against mycelial growth of fungal pathogens at $P<0.05$. At 50 mg/mL extract concentration, radial growth (cm) recorded in *L. theobroamae*, *C. muse*, *C. asianum* and *F. longipes* were 16.00 ± 1.00, 10.33 ± 1.53, 8.33 ± 0.58 and 15.33 ± 1.53 mm respectively (Table 2), while percentage radial growth inhibition (% PIRG) caused by the treatments were 80.08 ± 1.25, 34.18 ± 9.73, 70.58 ± 5.09 and 20.67 ± 7.90 % respectively for *L. theobroamae*, *C. muse*, *C. asianum* and *F. longipes* Table 3. Under treatment with 70 mg/mL leaf extract, *L. theobroamae* recorded radial growth of 8.67 ± 0.73 mm, *C. muse* 0.00 mm, *C. asianum* 0.00 mm, and *F. longipes* showed 9.00 ± 1.00 mm radial growth respectively. From the Table 3, pathogen growth inhibition showed 90.46 ± 0.72, 100.00 ± 0.00, 100.00 ± 0.00 and 53.44 ± 5.17 % growth inhibition was recorded in *L. theobromae*, *C. musae*, *C. asianum* and *F. longipes* respectively. The higest radial growth inhibition (100%) was achieved in all pathogens at 100 mg/mL extract concentration. The trend of inhibition was concentration dependent, thus, as leaf extract concentration was increased, pathogen growth inhibition also increased (Figure 1). Results in Table 2, shows that the two *Colletotrichum species* were the most sensitive organisms to the extract, hence completely inhibited at 70 mg/mL extract concentration, followed by *Fusarium longipes* at 90 mg/mL and lastly *L. theobromae* at 100 mg/mL.

### SEM Microscopy of Antifungal Activities of 50, 60, 70, and 80 mg/mL Concentration *M. Citrifolia* Leaf Extract in PDA Media Against *C. Musae*

SEM image of *C. musae* isolates growing on 50, 60, 70, and 80 mg/mL of *M. citrifolia* extract amended PDA showed severe distortion of fungal hyphae. Microscopic observation showed

severe hyphal distortion, cell collapsed/cell lysis and complete destruction after 7 days of incubation Figure 2 (a-d) while (e) the control remained intact. Hyphal distortion increased with an increment of extract concentration.

## DISCUSSION

GC-MS chromatogram of *M. citrifolia* leaf extract revealed that the extract is a mixture of several volatile bioactive compounds. Analysis showed up to 67.51% of the total volatile compounds contained strong antimicrobial compounds in the form of Phytol (Diterpene), All-trans-Squalene (Triderpene), 2H-1-Benzopyran-6-ol (Vitamin compound), Isobutylglycerol nitro (polyphenol), Linolenic acid (fatty acid), Pyran-4-one (Flavonoid), Palmitic acid, 3-oxo-1-butenyl (phenol), and Gama- Sitosterol (Steroids), Phytol, (2- Hexadecen-1-ol) alone constituted 25.96% of the total bioactive compounds, while squalene; a precursor of steroids constituted 15.13% of the *M. citrifolia* leaf extract.

**Table 1: GC-MS analysis of *M. citrifolia* leaf phytochemical compounds with their biological activities**

| Compounds' name | RT | MW | %Peak height | MF | Compound class | Biological activities & references |
|---|---|---|---|---|---|---|
| Phytol 2- Hexadecen-1-ol, (3,7,11,15-tetramethyl-) | 22.040 | 296 | 25.96 | $C_{20}H_{40}O_2$ | Diterpene | Antimicrobial, anticancer, antinflammatory (Saravanan *et al.*, 2014). |
| All-trans-Squalene (2,6,10,14,18,22-Tetracosahexaene), | 27.723 | 410 | 15.13 | $C_{30}H_{50}$ | Triterpene | Antibacterial, antioxidant, chemopreventive, (pesticide) (Jegajeevanram *et al.*, 2014) |
| 2H-1-Benzopyran-6-ol, (Vitamin E) | 30.344 | 430 | 5.14 | $C_{29}H_5O_2$ | Vitamin compound | Analgesic, Antiinflammatory, Antioxidant, Antidermatitic, antitumor, hepatoprotective, (Zekeya *et al.*, 2016) |
| 1,3-Propanediol, (Isobutylglycerol nitro) | 14.734 | 151 | 4.68 | $C_4H_9NO_5$ | Polyphenol | Antimicrobials, antioxidant activities, (Khan *et al.*, 2017; Yassin, 2017) |
| Linolenic acid, (alpha-Linolenic acid) | 22.280 | 278 | 4.20 | $C_{18}H_{30}O_2$ | fatty acid | Flavouring agent, perfumes, Ice-cream (Kumar *et al.*, 2014) |
| 5-Dihydro-6-methyl-3, (Pyran-4-one) 4H- | 10.463 | 144 | 3.83 | $C_6H_8O_4$ | Flavonoid | Antimicrobial, anturmor, and for cancer treatment. (Prakash *et al.*, 2009) |
| n-Hexadecanoic acid (Palmitic acid) | 20.572 | 256 | 3.59 | $C_{16}H_{32}O_2$ | Palmitic acid | Antioxidant, Hypocholesterolemic Nematicide, (Kumar *et al.*, 2014) |
| 2-Cyclohexan-1-one, (3-oxo-1-butenyl) | 18.906 | 222 | 2.54 | $C_{13}H_{18}O_3$ | Phenols | Antimicrobial, insecticidal, antioxidant antibiotics. (Sabithira & Udayakumar, 2017). |
| Gama- Sitosterol (Stigmast-5-en-3-ol,) | 32.663 | 414 | 2.32 | $C_{29}H_{50}O$ | Steroids | Pain killer and Jaundice (Kumar *et al.*, 2014). |
| Maltol, (Larixinic acid) | 9.263 | 126 | 1.77 | $C_6H_6O_3$ | Pyranones | Antibacterial, antioxidant, flovour in food and cosmetics (Sabithira & Udayakumar, 2017). |
| Tetrahydro-3-furamethanol (Tetrahydro-3-furanylmethanol) | 16.515 | 102 | 1.67 | $C_5H_{10}O_2$ | Phenolic | Antioxidant, antimicrobial, anticancer, antiallergy therapy for asthma and vaccine adjuvants (Saravanan *et al.*, 2014). |
| Stigmasterol, (Stigmasta-5,22-dien-3-ol,) | 31.869 | 412 | 1.59 | $C_{29}H_{48}O$ | Stearic acid | Hepatoprotective (Kumar *et al.*, 2014). |
| Cyclohexanemethanol (3-methyl-1-butenyl) | 18.946 | 212 | 1.16 | $C_{13}H_{24}O_2$ | Benzyl alcohol | Antimicrobial, inhibition of synthesis of DNA and RNA in both fungi and bacteria (Hashim *et al.*, 2016) |
| 3-Deoxy-d-mannanic acid | 16.874 | 180 | 1.09 | $C_6H_{12}O_6$ | Glucose | unknown |
| Ergost-5-en-3-ol, (Ergost-5-en-3. beta-ol) | 31.566 | 400 | 1.02 | $C_{28}H_{48}O$ | Steroids | Antimicrobial, anticancer, antinflammatory, Hepatoprotective (Kumar *et al.*, 2014). |

Key: RT=Retention time, MW=Molecular weight, MF=Molecular formula

**Table 2: Antifungal activities of 50, 60, 70, 80, 90, and 100 mg/mL *M. citrifolia* leaf extract against colony diameter (radial growth) and interaction effect between leaf extract (E) and pathogens (P) after 72 hrs of incubation at 25±2 °C and 80-85% RH**

| Extract con. (E) (mg/mL) | Colony growth diameter (mm) after 72 hrs | | | |
|---|---|---|---|---|
| | *L. theobromae* | *C. musae* | *C. asianum* | *F. longipes* |
| Control | 80.33±1.53a | 15.67±2.08a | 11.33±1.53a | 19.33±2.52a |
| 50 | 16.00±1.00b | 10.33±1.53b | 8.33±0.58b | 15.33±1.53b |
| 60 | 14.00±1.0c | 4.67±0.58c | 5.33±0.65c | 11.67±2.08c |
| 70 | 8.67±0.73d | 0.00±0.00d | 0.00±0.00d | 9.00±1.00d |
| 80 | 5.67±0.58e | 0.00±0.00d | 0.00±0.00d | 7.00±2.65e |
| 90 | 4.33±0.62e | 0.00±0.00d | 0.00±0.00d | 0.00±0.00f |
| 100 | 0.00±0.00f | 0.00±0.00d | 0.00±0.00d | 0.00±0.00f |
| E*P | *** | *** | *** | *** |

Means followed by the same letter in the same column are not significantly different at P=0.05. Significant means were separated by Turkey, *** = highly significant at P<0.05. (n=3). Key: E=Extract and P=pathogens. Error interval=0.45≤x < 0.55

Table 3: Mean effect of 50, 60, 70, 80, 90, and 100 mg/mL concentrations of *M. citrifolia* leaf extract amended PDA media on percentage inhibition of pathogen radial growth (% PIRG) and interaction effect between leaf extract (E) and pathogens (P) after 72 hrs of incubation at 25±2 °C and 80-85% RH

| Extract conc. (E) (mg/mL) | Percentage inhibition of pathogen radial growth (% PIRG) after 72 hrs | | | |
|---|---|---|---|---|
| | *L. theobromae* | *C. musae* | *C. asianum* | *F. longipes* |
| Control | 0.00±0.00d | 0.00±0.00e | 0.00±0.00d | 0. 00±0.00f |
| 50 | 80.08±1.25c | 34.18±9.73c | 70.58±5.09c | 20.67±7.90e |
| 60 | 82.57±1.25c | 70.28±3.68b | 79.41±5.1 0b | 39.64±10.77d |
| 70 | 90.46±0.72b | 100.00±0.00a | 100.00±0.00a | 53.44±5.17c |
| 80 | 92.95±0.72b | 100.00±0.00a | 100.00±0.00a | 63.79±8.68b |
| 90 | 94.61±0.72b | 100.00±0.00a | 100.00±0.00a | 100.00±0.00a |
| 100 | 100.00±0.00a | 100.00±0.00a | 100.00±0.00a | 100.00±0.00a |
| E*P | *** | *** | *** | *** |

Means followed by the same letter (s) in the same column are not significantly different at P=0.05. Significant means were separated by Turkey.
*** = highly significant at P<0.05, (n=3)

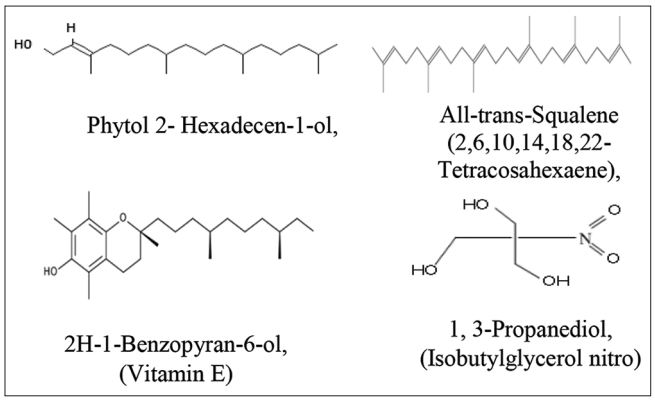


**Figure 1:** Names and structures of the identified principal phytochemicals compounds that constitutes 50.91% of the total volatile bioactive constituents of *M. citrifolia* using GC-MS technique

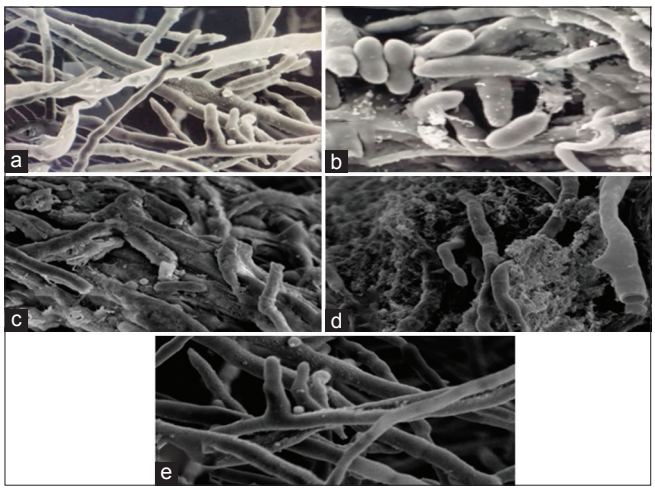


**Figure 2:** SEM images of antifungal activities of different concentrations (a) 50, (b) 60, (c) 70, and (d) 80 mg/mL of *M. citrifolia* leaf extract against *C. musae* and (e) *C. musae* grown on plate without leaf extract (control). Photos were taken 7 days after the treatment at 1,500 magnification

The findings of this study are in conformity with reports of previous studies reported by Wang and Su (2001), Sang *et al.* (2002) and Krishnaiah *et al.* (2012), who reported that the major bioactive compounds of *M. citrifolia* leaf are phenolic compounds, alkaloids and organic acids. Similarly, Assi *et al.* (2017), documented over 200 phytochemicals isolated and identified from various parts of *M. citrifolia* plant, and that *M. citrifolia* leaf extract was antimicrobial, anticancer, larvicidal and antioxidant in nature. According to Kakad *et al.* (2015), phenol, tannin, alkaloid, glycosides, flavonoid, terpenoids and steroids were isolated from *M. citrifolia* leaf extract. Bharathy *et al.* (2012) reported phytol, as diterpene with significantly strong antimicrobial activities against many bacterial and fungal strains. Zin *et al.* (2007) reported existence of phenolic and flavonoids components of *M. citrifolia* leaves constitute 1,095 ± 0,241 mg/g GAE and 0.0483 mg/g EQ respectively. However, Assi *et al.* (2017) reported the strongest inhibitory effect of methanol extract of *M. citrifolia* leaf (79.8 %) against different fungal pathogens. Wang and Su (2001), Kurniawan (2018) and Setyani and Setyowati (2018) and reported the major phytochemicals in *M. citrifolia* are phenolic compounds, organic acids, and alkaloids.

Based on the findings of the present study, researcher reports the significant inhibitory effect of the methanolic extract of *M. citrifolia* leaf ($P< 0.05$) on fungal growth, which was concentration dependent. Furthermore, the results on the antimicrobial properties of *M. citrifolia* in this study were in agreement with the report of Kakad *et al.* (2015) who reported the antifungal activities of methanol-ethanol leaf extract of *M. citrifolia* against *Daedalea flavida*, *Candida albicans and Aspergillus niger*. Sundrarajan *et al.* (2017) also found a superior antimicrobial properties of *M. citrifolia* leaf extract against human pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans*.

Furthermore, previous studies by Jagtap *et al.* (2009), Idris *et al.* (2015) and Bhutia *et al.* (2016) reported a highly inhibitory effect of plant extract and essential oils on mycelial growth of *Colletotrichum musae* and further stated that the inhibition was directly dependent on the quantity of extract added to the growth medium. However, each fungal isolate in the present study showed different levels of susceptibility to the extract, hence resulting in different MIC for each fungus. Implying *Colletotrichum* species were the most susceptible fungal species to the extract followed by *F. longipes* and *L. theobromae*.

## CONCLUSION

Seventeen major volatile bioactive constituents were identified from the extract of *M. citrifolia*. The identified compounds were found to have strong antifungal properties against crown rot inciting pathogens; *L. theobromae*, *C. musae*, *C. asianum*, and *F. longipes* on PDA media. The presence of tannins, steroids, saponins, flavonoids and alkaloids in the leaf extract established through GC-MS analysis of the extract further confirmed the presence of strong potent bioactive compounds, principal among them were diterpene, triterpene, alkaloids, phenolic compounds with their derivatives, flavonoids, steroids, tannins, organic acids, and some vitamins. Hence, this study has evidently indicated the strong antifungal properties of *M. citrifolia* properties that can be exploited and used as safe alternatives to the use of synthetic fungicide for the control of crown rot inciting pathogens.

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