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# ASSESSMENT OF PLANTS' LEAF EXTRACTS FOR ANTIFUNGAL ACTIVITY AGAINST THE FUNGUS *COLLETOTRICHUM ACUTATUM* IN CHILI (*CAPSICUM ANNUUM* L.)

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## SUMMARY

In horticultural crops, including chili, the wide use of synthetic fungicides has manifested to control anthracnose disease; however, these chemicals have some side effects to deal with. In reducing the dependency on synthetic fungicides, other alternatives and means need searching to prevent pathogenic fungi. Therefore, the presented study sought to examine plant extracts with the potential to inhibit the growth of pathogenic fungi *Colletotrichum acutatum,* the causal organism of anthracnose disease in chili. This study collected 20 potential plant species for the investigation. The leaf extraction used the maceration method in methanol and n-hexane. The contents of chemical compounds sustained the GC-MS analysis. All the leaf extracts tested for their bioactivity underwent the colony method and diffusion well. The results revealed that out of 20 types of plants, six plant species were capable of inhibiting the growth of *C. acutatum* fungi, including *Piper nigrum, Piper ornatum, Piper retrofractum, Ficus septica, Samanea saman,* and *Tithonia diversifolia*. The leaf extract of *F. septica* has the highest inhibition rate (81.11%) for the growth of *C. acutatum* compared with other plant leaf extracts. The GC-MS analysis of the *F. septica* leaf extract showed the presence of 15 types of metabolite constituents, with nine having antimicrobial activities.

**Keywords:** *Colletotrichum acutatum*, anthracnose, synthetic fungicides, side effects, botanical fungicide, leaf extract, antimicrobial properties, *Ficus septica*

**Key findings:** This latest study found plant extracts that have the potential to inhibit the growth of the pathogenic fungi *Colletotrichum acutatum,* which caused anthracnose disease in crop plants.

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## INTRODUCTION

Chili (*Capsicum annuum* L.) is a popular horticultural plant and spice in Asia, mainly used to create spicy hints in Asian cuisine. In Indonesia, especially in Bali, chili has been highly produced and traded because of its highest demand in the market. According to the Indonesia Central Bureau of Statistics (2022) data, chili productivity has been declining by 5.67% in the last two years in Bali, Indonesia. Several factors are responsible for this decline in chili productivity, one of which is critically a pathogenic fungus that caused a particular disease.

Anthracnose is a plant disease that mainly infects horticultural crops, including chili. Past studies reported that anthracnose is the foremost disease in chili caused by the fungus *Colletotrichum* spp. (Aziziy *et al.*, 2020). Other fungi, such as *Colletotrichum,* i.e., *Colletotrichum gloeosporioides, C. capsica, C. demantium,* and *C. cocodes,* can also cause the disease (Sari and Rina, 2021). However, in Bali, Indonesia, *Colletotrichum acutatum* typically triggers anthracnose disease in chili (Sudirga, 2016). According to Mariana *et al.* (2021), the anthracnose disease occurs during the rainy season, reducing the crop yield by 50% to 100%.

For anthracnose disease control in chili, synthetic fungicides with active substances include manganese ethylene bis dithiocarbamate, azoxystrobin, trifloxystrobin, and pyraclostrobin (Katediya *et al*., 2019). Continuous use of synthetic fungicides with inappropriate doses can cause several side effects harmful to human health and the environment (Johnny *et al.*, 2011). Therefore, replacing synthetic fungicides with an alternative like botanical fungicides is necessary, which can also suppress and inhibit the growth of fungi and diseases in cultivated plants. For this approach to develop the botanical fungicides, several potential plant species require scrutiny for their antifungal activities (Bhandari *et al*., 2021).

The prior study has reported that six plant species tested for their antifungal activity against the fungus *C. gloeosporioides,* which also causes anthracnose disease in papaya plants, and found out the extract of *Lantana camara* provides the highest inhibition (90.71%) for *C. gloeosporioides* growth (Dissanayake *et al*., 2019). Suprapta and Khalimi (2012) reported that suar (*Albizia saman*) leaf extracts showed the maximum antifungal activity among the 14 plant species tested for their antifungal activities against the fungus that causes Fusarium wilt in peppers. Meanwhile, out of 19 plant species, ethyl acetate extracts of *Lantana camara* showed the highest antifungal activity and inhibition rate (88.70%) against the fungus *C. gloeosporioides* causing the anthracnose disease in papaya (Ademe *et al*., 2013). The relatable study's probe on the antifungal activity of several leaf extracts of local plants commenced to judge their potential in inhibiting the fungus *C. acutatum* growth that causes anthracnose in chili crops. Several local plants in Bali, especially from the families Zingiberaceae, Asteraceae, Moraceae, and Piperaceae, contain active compounds that have the potential to act as fungicides.

## MATERIALS AND METHODS

### Plant collection and leaf extraction

Plant extracts have the potential to inhibit the growth of pathogenic fungi *Colletotrichum acutatum,* which is the causal organism of anthracnose disease in horticultural crops, including chili. Twenty different plant species, collected through explorative methods, came from the four diverse areas of Bali, Indonesia. The selected species potentially have antifungal activities, as shown in Table 1. The basis for species collection was the local knowledge of the plants as a component in traditional medicine. For screening of their antifungal activities, sliced leaves of all the 20 collected species into smaller pieces received heat. After drying, the sliced leaves attained pulverization using a blender. The powder then sustained maceration with methanol PA (pro-analysis) and n-hexane for 72 h to extract the bioactive compounds found in the plant's leaves, following the protocols from Zana *et al.* (2021). Meanwhile, the succeeding steps used

**Table 1.** The plant species used in the antifungal activity test. The scientific name of the species checked through https://plantsoftheworldonline.org/ for the latest accepted name.

| No. | Family | Species | Local Name |
|---|---|---|---|
| 1 | Annonaceae | *Annona squamosa* L. | Srikaya |
| 2 | Apocynaceae | *Allamanda cathartica* L. | Alamanda |
| 3 | Apocynaceae | *Plumeria rubra* L. | Kamboja, jepun |
| 4 | Asteraceae | *Ageratum conyzoides* L. | Babandotan |
| 5 | Asteraceae | *Sphaeranthus indicus* L. | Sembung delan |
| 6 | Asteraceae | *Tithonia diversifolia* (Hemsl.) A.Gray | Kembang bulan, paitan |
| 7 | Fabaceae | *Cassia fistula* L. | Trengguli |
| 8 | Fabaceae | *Dalbergia latifolia* Roxb. | Sonokeling |
| 9 | Fabaceae | *Samanea saman* (Jacq.) Merr. | Trembesi |
| 10 | Meliaceae | *Azadirachta indica* A.Juss. | Intaran, mimba |
| 11 | Meliaceae | *Melia azedarach* L. | Mindi kecil, gempinis |
| 12 | Meliaceae | *Swietenia mahagoni* (L.) Jacq. | Mahoni |
| 13 | Menispermaceae | *Tinospora crispa* (L.) Hook.f. & Thomson | Brotowali |
| 14 | Moraceae | *Ficus septica* Burm.f. | Awar-awar |
| 15 | Plantaginaceae | *Plantago major* L. | Daun sendok, ki urat |
| 16 | Piperaceae | *Peperomia latifolia* Miq. | Sasabahan gunung |
| 17 | Piperaceae | *Piper nigrum* L. | Lada, merica |
| 18 | Piperaceae | *Piper ornatum* N.E.Br. | Sirih merah |
| 19 | Piperaceae | *Piper retrofractum* Vahl | Cabai jawa |
| 20 | Zingiberaceae | *Curcuma longa* L. | Kunyit, kunir |

the protocol developed by Suprapta and Khalimi (2012). The macerated powder went through four layers of gauze and a Whatman No.1 filter paper for filtering. Afterward, the filtrate's evaporation used a vacuum rotary evaporator at 40 °C to obtain the crude extract of the plant leaves. The resulting distillate of each species incurs testing with the *C. acutatum* isolate.

**Pathogenic fungal isolate sources**

Pathogenic fungal isolates of *C. acutatum* came from the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Udayana University, Kuta Selatan, Bali, Indonesia.

**Antifungal activity**

Antifungal activity test of 20 plant species' crude extracts against the *C. acutatum* progressed using the well-diffusion method. Petri dishes earlier filled with 10 mL of the PDA media and 200 µL of fungal spores of the fungus isolate *C. acutatum* were allowed to solidify. After solidification, creating diffusion wells used a cork borer with two diffusion wells in each Petri dish. Then, each diffusion well acquired 20 µL of the crude extract in the Petri dishes. According to Yang *et al*. (2019), if the inhibition zone is more than 20 mm, it means the resistance is very strong; if the inhibition zone is 10–20 mm, the resistance is strong; if the inhibition zone is 5–10 mm, the resistance is moderate; and if less than 5 mm, the resistance is weak. The leaf extracts that can form an inhibition zone against *C. acutatum* isolate gained preparation for inhibitory test through the Minimum Inhibitory Concentration (MIC) and colony method.

***Minimum inhibitory concentration (MIC)***

Determining the value of the MIC in the crude leaf extract had the study carry out the well-diffusion method. The treatments of raw extract concentrations were 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, and 5%, with a sterile distilled water (0%) used as a control. The research used a completely random design with 15 treatments and three replications (n = 45 for each extract).

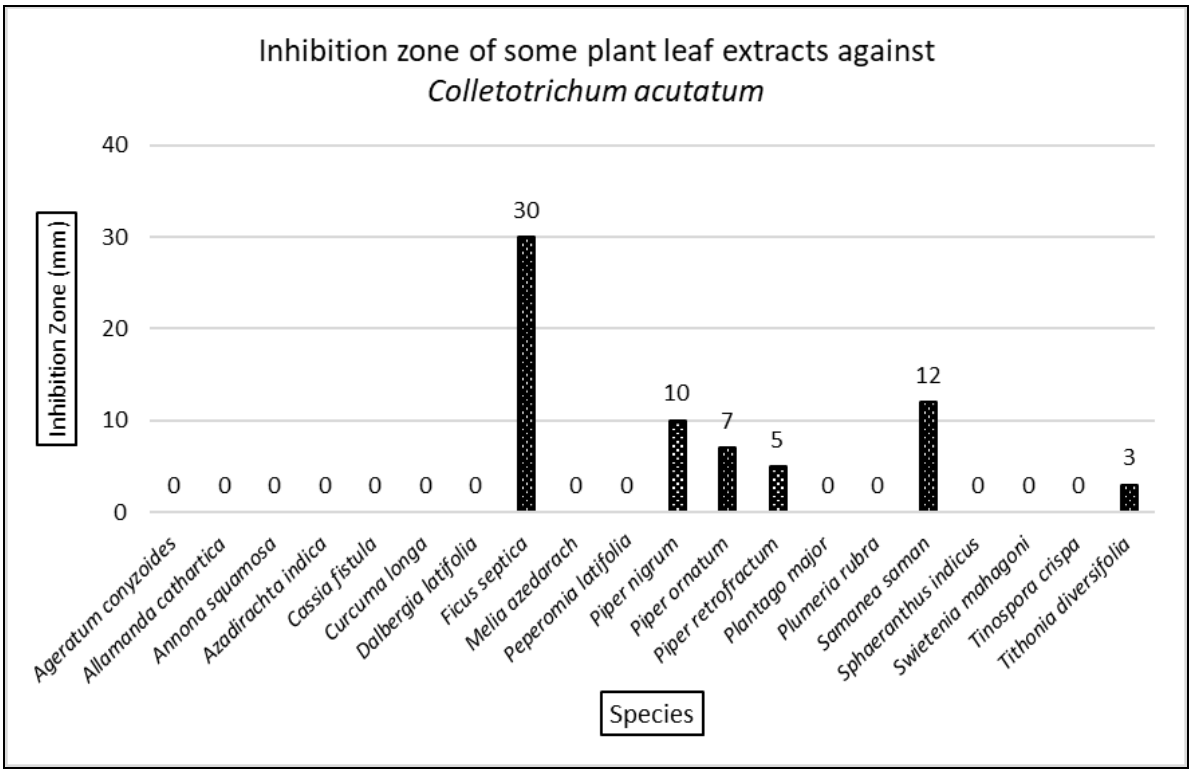


**Figure 1.** Inhibition zone of some plant leaf extracts against *Colletotrichum acutatum.*

**Identification of active compound**

In plant leaf extracts, identifying the active substance for best inhibition against the fungus *C. acutatum* continued using Gas Chromatography-Mass Spectroscopy (GC-MS QP2010 Ultra Shimadzu). The GC-MS analyzed extremely active and relatively pure fractions. The eluent used was MeOH/H2O with a ratio of 40:60, Wakosil ODS/5C18-200 column, size 4.6 mm × 200 mm, eluent flow rate of 1 mL/minute, temperature of 250 °C, and detection using UV light at 254 nm. Carrying out the detection results employed matching the molecular weights and fragmentation patterns of the isolated compounds with those in the GC-MS library so that the molecular weight and identified compounds' molecular structure could be distinguishable.

**RESULTS**

**Plants screening for antifungal activity**

The results of the antifungal activity tests of the 20 plant species against the fungus *C. acutatum* appear in Figure 1. Based on the obtained data, out of 20 plant species tested, six plant species (*Ficus septica, Samanea saman, Piper nigrum, Piper ornatum, Piper retrofractum*, and *Tithonia diversifolia*) manifested to inhibit the growth of the fungus *C. acutatum*. The varied inhibition zones of these six species were *Ficus septica* (30 mm inhibition zone), *Samanea saman* (12 mm inhibition zone), *Piper nigrum* (10 mm inhibition zone), *Piper ornatum* (inhibition zone of 7 mm), *Piper retrofractum* (5 mm inhibition zone), and *Tithonia diversifolia* (3 mm

inhibition zone). The extract of *F. septica* became the extract with the highest inhibition zone. It can be due to the extracted secondary metabolites that have antimicrobial activity. The estimation of the extract efficiency showed the Minimum Inhibitory Concentration (MIC). The MIC test results of the six plant species against the fungus *C. acutatum* used the well-diffusion method, as presented in Table 2.

**Minimum inhibitory concentration (MIC) and colony test**

The MIC value of the six plant species tested against the fungus *C. acutatum* revealed varied responses (Table 2). The species *Ficus septica* had the lowest MIC value of 0.9% with an inhibition zone of 7.25 ± 0.05 mm, followed by *S. saman* 1% (4.56 ± 0.15 mm), *P. ornatum* 2% (6.33 ± 0.13 mm), *P. nigrum* 2% (5.22 ± 0.05 mm*), P. retrofractum* 3% (7.88 ± 0.16 mm), and *T. diversifolia* 3% (5.80 ± 0.15 mm). The MIC showed the lowest concentration that can inhibit *C. acutatum*, with the extract of *F. septica* having the best MIC at 0.9% of extract concentration. The inhibition diameter gets broader when the concentration increases, making the 5% extract concentration have the highest MIC value for each species. Ensuring the accuracy of the inhibition, other tests, such as the colony test, also proceeded. The inhibition test results of the six different plant species, i.e., *F. septica, P. nigrum, P. ornatum, P. retrofractum, S. saman*, and *T. diversifolia* leaf extracts on the growth of the fungus *C. acutatum* using the colony test method are available in Table 3.

Based on the results, the higher the concentration of leaf extracts, the smaller the diameter of the *C. acutatum* colony (Table 3). Of the six plant species tested, *F. septica* leaf extract showed the highest and most intense inhibition, followed by S*. saman, P. nigrum, P. ornatum, P. retrofractum,* and *T. diversifolia*. The species *Ficus septica* leaf extract concentration of 5% provided the maximum inhibition, as indicated by the tiniest *C. acutatum* colony (17 mm in diameter). Even for the 1% extract concentration, the extract of *F. septica* displayed more than 27 mm colony reduction, and the other five species exhibited less than 10 mm colony reduction when compared with the 0% extract concentration. The ability of *F. septica* leaf extract to inhibit the growth of *C. acutatum* is due to the presence of secondary metabolites contained therein. In the species *F. septica*, the leaf extract content of secondary metabolites occurs in Figure 2 and Table 4.

**DISCUSSION**

Plant leaf extracts have different abilities in inhibiting the growth of pathogenic fungi *C. acutatum*. Among the 20 plant species tested on the *C. acutatum*, six plant species, viz., *F. septica, P. nigrum, P. ornatum, P. retrofractum, S. saman*, and *T. diversifolia,* showed antifungal activity against its growth*.* Among those six species, the crude leaf extract of *F. septica* demonstrated the maximum inhibition against *C. acutatum* with 30 mm of inhibition diameter. Johnny *et al.* (2011) tested the antifungal activity of 15 types of plant extracts against the fungus *C. capsici,* and they found that five types of plants could inhibit the growth of the tested fungi with an inhibition zone above 40 mm. These five plant species were *P. betle, Alphinia galanga, Centella asiatica, Momordica charantia,* and *P. minus,* with diameters of inhibition zones, i.e., 71.87, 62.78, 59.07, 48.32, and 46.92 mm, respectively.

The ability of plant extracts to inhibit the growth of pathogens is measurable by the minimum inhibition of the extracts. Minimum inhibitory concentration (MIC) is the lowest concentration that can inhibit the growth of pathogens. Results showed that the species *F. septica* leaf extract has the lowest MIC value (0.9%) against the fungus *C. acutatum* with an inhibition of 7.25 ± 0.05 mm. According to Sudirga (2016), the MIC value of a plant extract against a particular pathogen indicates the level of toxicity of the distillate against the tested pathogen. The smaller the concentration that inhibits the growth of *C. acutatum*, the greater the toxicity level of the extract to the test pathogen and vice versa. According to Krochmal and Wicher (2021), the differences in

**Table 2.** Diameter of inhibition (mm) based on extract concentration (%) to inhibit the *C. acutatum* in the Minimum Inhibitory Concentration (MIC) test.

| No. | Species | Extract Concentration (%) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1 | 2 | 3 | 4 | 5 |
| 1 | *Ficus septica* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7.25 ± 0.05 | 8.12 ± 0.08 | 9.33 ± 0.15 | 11.54 ± 0.08 | 14.78 ± 0.18 | 15.15 ± 0.17 |
| 2 | *Piper nigrum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.22 ± 0.05 | 7.51 ± 0.15 | 8.58 ± 0.20 | 9.78 ± 0.08 |
| 3 | *Piper ornatum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.33 ± 0.13 | 8.05 ± 0.16 | 9.78 ± 0.25 | 11.34 ± 0.19 |
| 4 | *Piper retrofractum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7.88 ± 0.16 | 8.90 ± 0.09 | 10.11 ± 0.18 |
| 5 | *Samanea saman* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.56 ± 0.15 | 6.55 ± 0.18 | 8.12 ± 0.21 | 10.38 ± 0.17 | 12.23 ± 0.23 |
| 6 | *Tithonia diversifolia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.80 ± 0.15 | 7.65 ± 0.21 | 8.95 ± 0.22 |

**Table 3.** Diameter of the colony (mm) based on extract concentration (%) to inhibit the *C. acutatum* in the colony method.

| No. | Species | Extract concentration (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | *Ficus septica* | 90.00 ± 0.00 | 63.25 ± 0.01$^{i}$ | 55.00 ± 0.00$^{d}$ | 47.75 ± 0.03$^{c}$ | 38.50 ± 0.03$^{b}$ | 17.00 ± 0.00$^{a}$ |
| 2 | *Piper nigrum* | 90.00 ± 0.00$^{y}$ | 82.24 ± 0.02$^{u}$ | 75.31 ± 0.21$^{r}$ | 70.28 ± 0.02$^{n}$ | 67.46 ± 0.03$^{l}$ | 60.72 ± 0.02$^{h}$ |
| 3 | *Piper ornatum* | 90.00 ± 0.00$^{y}$ | 80.12 ± 0.01$^{t}$ | 72.31 ± 0.01$^{p}$ | 70.43 ± 0.03$^{o}$ | 65.25 ± 0.02$^{j}$ | 58.82 ± 0.02$^{f}$ |
| 4 | *Piper retrofractum* | 90.00 ± 0.00$^{y}$ | 85.32 ± 0.01$^{w}$ | 78.78 ± 0.03$^{s}$ | 75.25 ± 0.02$^{r}$ | 70.38 ± 0.02$^{o}$ | 68.59 ± 0.02$^{m}$ |
| 5 | *Samanea saman* | 90.00 ± 0.00$^{y}$ | 80.15 ± 0.05$^{t}$ | 72.71 ± 0.03$^{q}$ | 67.25 ± 0.02$^{k}$ | 60.36 ± 0.02$^{g}$ | 55.53 ± 0.03$^{e}$ |
| 6 | *Tithonia diversifolia* | 90.00 ± 0.00$^{y}$ | 89.96 ± 0.06$^{y}$ | 87.52 ± 0.04$^{x}$ | 85.37 ± 0.03$^{w}$ | 82.65 ± 0.04$^{v}$ | 80.16 ± 0.04$^{t}$ |

The average value that followed with the same notation in column and row indicate insignificant value in DMRT (error degree of 5%).

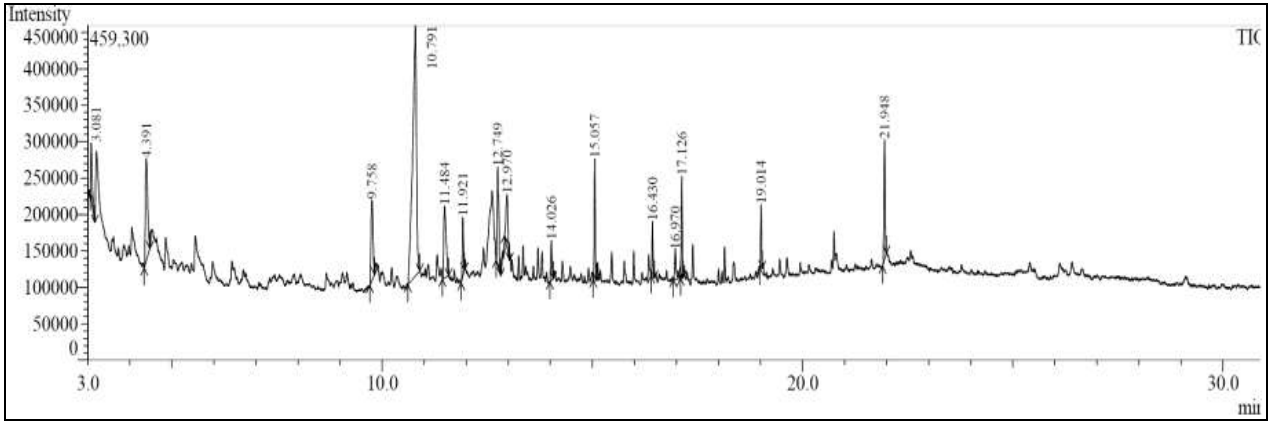


**Figure 2.** Chromatogram of the GC-MS analysis of the *F. septica* leaf extract with the highest inhibition against *Colletotrichum acutatum.*

**Table 4.** The identified bioactive compounds in the *F. septica* leaf extract that have the potential to inhibit the growth of *Colletotrichum acutatum*.

| No. | Peak | Molecule Weight | Molecule Formula | Retention Time (min) | Area (%) | Compound |
|---|---|---|---|---|---|---|
| 1 | Peak 1 | 180 | $C_6H_{12}O_6$ | 3.079 | 2.22 | dl-Glyceraldehyde |
| 2 | Peak 2 | 142 | $C_{10}H_{22}$ | 4.391 | 15.74 | Heptane |
| 3 | Peak 3 | 402 | $C_{23}H_{46}O_3S$ | 9.760 | 5.27 | Sulfurous acid |
| 4 | Peak 4 | 283 | $C_{10}H_{13}N_5O_5$ | 10.791 | 39.06 | Guanosine |
| 5 | Peak 5 | 180 | $C_6H_{12}O_6$ | 11.489 | 7.92 | D-Allose |
| 6 | Peak 6 | 214 | $C_{13}H_{26}O_2$ | 11.920 | 3.01 | Dodecanoic acid |
| 7 | Peak 7 | 222 | $C_{12}H_{14}O_4$ | 12.750 | 5.18 | 1,2-Benzenedicarboxylic acid |
| 8 | Peak 8 | 180 | $C_6H_{12}O_6$ | 12.972 | 4.444 | 3-Deoxy-d-mannonic acid |
| 9 | Peak 9 | 196 | $C_{14}H_{28}$ | 14.029 | 1.07 | Cyclohexane |
| 10 | Peak 10 | 322 | $C_{23}H_{46}$ | 15.057 | 1.57 | 9-Tricosene |
| 11 | Peak 11 | 270 | $C_{17}H_{34}O_2$ | 16.430 | 2.00 | Hexadecanoic acid |
| 12 | Peak 12 | 666 | $C_{18}H_{54}O_9SI_9$ | 16.976 | 1.14 | Octadecamethyl-cyclononasiloxane |
| 13 | Peak 13 | 396 | $C_{27}H_{56}O$ | 17.125 | 1.63 | 1-Heptacosanol |
| 14 | Peak 14 | 396 | $C_{27}H_{56}O$ | 19.015 | 1.37 | 1-Heptacosanol |
| 15 | Peak 15 | 278 | $C_{16}H_{22}O_4$ | 21.947 | 8.38 | 1,2-Benzenedicarboxylic acid |

the results of the MIC test can refer to the differences in the type of metabolite constituents and their concentration found in the plant leaf extracts. The species *Syzygium cordatum* tested on the fungus *Colletotrichum dematium* had a MIC value of 6.25 mg/mL when extracted with acetone, a MIC value of 3.13 mg/mL when separated with water, and a MIC value of 1.56 mg/mL when isolated with ethyl acetate. Meanwhile, *Allium sativum* extracted with water, ethyl acetate, and acetone tested on the fungus *C. dematium* had MIC values of 6.25, 3.13, and 0.78 mg/mL, respectively.

The increase in extract concentrations tends to increase the inhibition zone. However, in specific circumstances, increasing the extract concentration causes a decrease in the inhibition zone. It can be because of several possibilities, one of which is a change in media pH due to an upsurge in extract concentration. Changes in media pH will indirectly affect the effectiveness of active compounds, which ultimately cause a decrease in the inhibition zone.

The colony test method is one of the best methods to assess the optimal inhibition of plant extracts against pathogens. The colony test method can show the optimal ability of plant extracts to inhibit the growth of pathogenic fungal colonies by measuring the diameter of the fungal colonies. Based on the existing results, the species *F. septica* leaf extract (5% concentration) was able to inhibit the growth of *C. acutatum* colonies (81.11%), which was significantly ($P \leq 0.5$) different compared with other treatments. Darmadi *et al.* (2017), using cinnamon leaf extract (*Cinnamomum burmanii*), found that the 0.9% optimal concentration of the extract inhibited the growth of the *Fusarium solani* colony by 53.08%. The ylang-ylang (*Cananga odorata*) flower extract (3% concentration) inhibited the growth of *C. acutatum* colonies by 22.93% for 10 days (Agung *et al.*, 2022).

The ability of the species *F. septica* to inhibit the growth of *C. acutatum* is due to bioactive compounds found in its leaf extract. Fifteen active compounds were prominent in the species *F. septica* leaf extract including dl-glyceral-dehyde, heptane, sulfurous acid, guanosine, d-allose, dodecanoic acid, 1,2-benzenedicarboxylic acid, 3-deoxy-d-mannonic acid, cyclohexane, 9-tricosene, hexadecanoic acid, octadecamethyl cyclononasiloxane, heptacosanol dan 1, and 2-benzenedicarboxylic acid. The ethanol and hexane fractionations found in *F. septica* leaf extract have the potential as the source of antimicrobial compounds. Castillo *et al.* (2012) also reported that *F. septica* contains bioactive compounds, antofine and ficuseptine. The antofine

compound has the potential as an anticancer compound, while the ficuseptine has the potential as an antibacterial and antifungal compound. Nugroho *et al.* (2013) revealed that the leaves, fruit, bark, and roots of *F. septica* contain alkaloids, saponins, and flavonoids, which are promising antimicrobial compounds.

Active compounds found in *F. septica* leaf extracts have been recorded to have antimicrobial properties, especially as antifungal and antibacterial. Past studies have also reported potential compounds, such as decane and hexadecanoic acids, found in *F. septica*. Akpuaka *et al.* (2013) investigated the biological activities of the neem (*Azadirachta indica*) extract and Skanda and Vijayakumar (2021) studied the antimicrobial activity of *Aspergillus arcoverdensis* extract. Decane is an alkane hydrocarbon commonly found in gasoline (in small quantities), and hexadecanoic acid is an ester compound (methyl ester) often called palmintic acid. Both compounds have antimicrobial activity, especially as antibacterial and antifungal (Akpuaka *et al.*, 2013; Skanda and Vijayakumar, 2021; Jasmi *et al.,* 2023).

Dodecanoic acid, known as lauric acid, a compound in the methyl ester group, has antimicrobial activity (Nakatsuji *et al.*, 2009; Carolina *et al.*, 2011). Lauric acid could inhibit the growth of pathogenic bacteria (*Propionibacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis, Mycobacterium tuberculosis,* and *Streptococcus pneumonia*), pathogenic fungi (*Candida albicans, Aspergillus niger,* and *Fusarium* spp.), and could also perform antiviral activity in human infections (Nitbani *et al.*, 2022). The effectiveness of lauric acid as an antimicrobial compound also known as a non-toxic natural product that is safe to humans raised the benefits of lauric acid for use in crop plants as botanical pesticide (Jin *et al.*, 2021).

The other ester compounds, also obtained in the species *F. septica* leaf extracts, comprised the 1,2-benzenedicarboxylic acid (mono ethylhexyl phthalate) and 3-deoxy-d-mannonic acid (uronic acid). The uronic acid is also distinct for antifungal activity (Martinez *et al.*, 2009), while the 1,2-benzenedicarboxylic acid has more complex biological activity, such as antifungal, antimicrobial, antioxidant, and anticancer (Raman *et al.*, 2012; Zayed *et al.*, 2019; Kumar *et al.*, 2022a). The 1,2-benzenedicarboxylic acid is also beneficial as an effective biopesticide (as larvicidal and pupicidal) in mosquito control, especially for *Culex quinquefasciatus* and *Aedes aegypti* (Kumar *et al.*, 2022b).

Sulfuric acid compounds, commonly used as antimicrobial desiccants in medicine (Lupse *et al.*, 2021), in higher plants, they are antifungal agents (Mazid *et al.*, 2011). The 1-heptacosanol is also an essential chemical compound vital in protecting from pathogenic fungal infections (Chowdhary and Kaushik, 2018; Hawar *et al.*, 2023). Raman *et al.* (2012) reported that 1-heptacosanol has biological activity as an anti-nematode, anticancer, antioxidant, and antimicrobial. Skanda and Vijayakumar (2021) also obtain 1-heptacosanol in the mycelia extract of *Aspergillus arcoverdensis* as an antimicrobial compound with antioxidative capacity.

The essential oil obtained in the extract of the species *F. septica* is octadecamethyl cyclononasiloxane, a volatile fatty acid ester abundant in *Jatropha* seed with antimicrobial activity (El-Din *et al.*, 2022). This compound also occurs in other parts of plants, such as in rhizome extracts of *Tectaria coadunata* (Dubal *et al.*, 2013) and in flower extracts of *Cassia fistula* (Ferdosi *et al.*, 2021). All past studies also confirmed the antimicrobial activity of octadecamethyl cyclononasiloxane.

Several researchers also worked on the *F. septica* leaf extract. Ueda *et al.* (2009) found new alkaloids from methanol extracts of leaf *F. septica,* aminocaprophenone and pyrrolidine; Vital *et al.* (2010) studied the antimicrobial activity along with the cytotoxicity and phytochemical screening; Nugroho *et al.* (2011) observed the cytotoxicity of *F. septica* leaf extract to breast cancer T47D cell; Ragasa *et al.* (2016) identified the chemical constituent of dichloromethane extract of *F. septica*; and Sudirga and Suprapta (2021) observed the biological control of *F. septica* extract to the *C. acutatum* infection in chili through scanning electron microscopy (SEM) and transmission electron microscopy (TEM). These researchers

focused on specific constituents of *F. septica* extract or its antimicrobial/antifungal activity. This presented study provides both the antifungal activity and the potential secondary metabolites derived from methanol and n-hexane extract of the *F. septica* leaf.

## CONCLUSIONS

Out of 20 plant species, six species' leaf extracts showed antifungal activity against *C. acutatum*. These six plant species were *Ficus septica, Piper nigrum, Piper ornatum, Piper retrofractum, Samanea saman*, and *Tithonia diversifolia*. Among the six, the leaf extract of *F. septica* showed the highest inhibitory for *C. acutatum* and contains various antifungal compounds.

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