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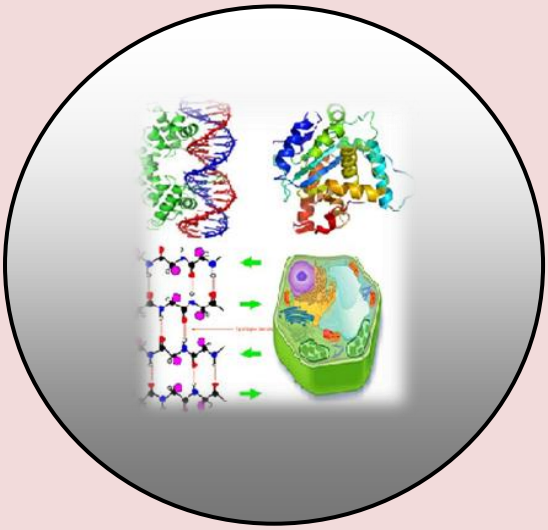

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RESEARCH PAPER

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ABSTRACT

Bioactive compounds of Mansoa alliacea leaf extract has potential as botanical pesticides to control Colletotrichum acutatum against anthracnose disease on chili pepper was be done. Base on in vitro test on PDA with inhibition zone diameter of 28 mm, but it is not certain bioactive compounds. For these problems, the aim is research is conducted to determine the content of bioactive compounds of Mansoa alliacea leaf extract to potentially as botanical pesticides. The method used for analysis of bioactive compaounds as column chromatography and thin layer, and GCMS. Base on phytochemicals test the extract of Mansoa alliacea containing 4 secondary metabolites are terpenoids, alkaloids, flavonoids, and phenols. Result analysis using GCMS there are 4 active compound namely pentan-1,3-dioldiisobutyrate, 2,2,4-trimetyl; tributyl acetylcitrate; terephthalic acid, di (2-ethylhecyl) ester and 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl. Based on the existing references, of 4 compounds and 3 of them have been known as antifungal compounds. Those are pentan-1,3-dioldiisobutyrate, 2,2,4-trimetyl; tributyl acetylcitrate; terephthalic acid, di(2-ethylhecyl) ester.

Keywords: Antifungal, Mansoa alliacea, Colletotrichum acutatum, Anthracnose Disease and Botanical Pesticides.

INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the potential horticultural commodities, because it has high economic value. Productivity of chili pepper is still low due to several factors; one of them is a factor of pests and diseases. Anthracnose in chili pepper is the most common disease and almost always occurs at planting area of chili pepper. According to Suryaningsih et al. (1996), the anthracnose pathogens most commonly found in chili pepper in Indonesia are the *Colletotrichum capsici* and *Colletotrichum gloeosporioides*. Anthracnosis besides causing a decrease in yield can also damage the aesthetic value of chili pepper. The decrease of yield due to anthracnose in chili pepper can reach 50% or more (Semangun, 2007). So far, to control anthracnose disease still relies on the use of synthetic fungicides. The continued use of synthetic fungicides can lead to the emergence of pathogenic resistance, pollute the environment and be harmful to consumers.

Based on this matter, it is necessary to find out of alternative to control anthracnose disease in chili pepper by utilizing plants that have the potential as botanical fungicides which are not harmful to consumers or the environment. According to Suprapta (2014) botanical pesticides have several advantages including botanical pesticides containing compounds of phenol, alkaloids, saponins, quinones, and xanthones which are easily biodegradable and not harmful to non-target organisms because they are specific to certain pests and pathogens. According to Nduagu et al. (2008) extract of *Ricinus communis*, *Azadirachta indica*, *Citrus limon*, *Psidium guajava*, *Hymenocardia acida* and *Cochlospermum planchonii*, can inhibit the growth of *Colletotrichum capsici* that cause anthracnose disease in chili pepper, with a diameter of the inhibition zone of 18, 7 mm, 16 mm, 15.7 mm, 15 mm, 13.4 mm and 9 mm. Whereas according to Johnny et al. (2011) extract of *Piper betle*, *Alpinia galanga*, *Centella asiatica*, *Momordica charantia* and *Polygonum minus* can inhibit the growth of *Colletotrichum capsici* with inhibition zone diameter of 71.87 mm, 62.78 mm, 59.07 mm, 48.32 mm and 46.92 mm respectively. According to Sudirga et al. (2014) crude extract of *Ficus septica* can inhibit the growth of *Colletotrichum acutatum* with an inhibition zone of 30 mm.

Mansoa alliacea (Figure 1) is an ornamental plant from the Bignoniaceae tribe originating from South America and has been widely planted in Indonesia as an ornamental plant. According to Chirunthorn et al. (2005) ethanol extract of *Mansoa hymenaea* leaves can inhibit the growth of *Candida albican* and *Trichophyton rubrum*. Whereas Towen et al. (2015) reported that *Mansoa alliacea* leaf extract had an effect on embryo formation and could inhibit tumor cell growth in mice. Base on primly study so that, crude extract of leaves of *Mansoa alliacea* able to inhibit the growth of *Colletotrichum acutatum in vitro* on PDA with inhibition zone diameter of 28 mm, but it is not certain bioactive substances. For these conditions, this study is a follow-up study, conducted to determine the content of bioactive substances potentially as botanical pesticides from leaves extract of *Mansoa alliacea*.

Figure 1. A = Plant of *Mansoa alliacea*, B= Anthracnose disease on chili pepper (Source: private collection, 2018)

MATERIALS AND METHODS

Methods of Extraction

Mansoa alliacea leaf extraction is done by drying the leaves at room temperature, then blending until it becomes powder. Then 100 grams of Awar leaves powder macerated with 1,000 ml of PA (Pro Analysis) methenol for 72 hours in a dark place and at room temperature. Then the extract was filtered with Whatman filter paper to obtain the filtrate. Then the filtrate was evaporated using a vaccum rotary evaporator (Iwaki, Japan) at a temperature of 40^0C until a crude extract was obtained, to obtain a crude extract that was used for further testing.

Antifungal Activity Test

Antifungal activity test of crude extract of the leaves of *Mansoa alliacea* against *Colletotrichum acutatum* was done in well diffusion method. According to Ardiansyah (2005), if the diameter of inhibition zone is ≥ 20 mm the inhibitory activity is very strong; 10-20 mm the inhibitory activity is strong; 5-10 mm the inhibitory activity is moderate; and ≤ 5 mm the inhibitory activity is poor or weak.

Analysis of Phytochemicals

Phytochemical analysis was conducted to determine the compound of the active fraction obtained by using reagents for specific classes of compounds. The compounds of the active components tested included: terpenoids, alkaloids, flavonoids, phenols, saponins, and tannins. Analysis was performed on fractions which showed the highest antifungal properties (Harborne, 1989).

Separation and Purification of Active Extracts

The crude extract of *Mansoa alliacea* was partitioned with n-hexane and methanol to obtain the extract phase of n-hexane and methanol phase. Furthermore, both the extracts were tested for the antifungal activity. The separation and purification was performed by column chromatography using silica gel (60 from 0.063 to 0.200 mm) as stationary phase, while the mobile phase is a mixture of various kinds of solvents which are based on differences in polarity. From the chromatography column, it produced several fractions and each fraction was tested for antifungal activity. Some active fractions were fractionated again using the same eluent as the previous fractionation. Each fraction obtained in the second fractionation was tested for antifungal activity and further active fractions were analyzed by KLT to determine the spot patterns generated from each of the fractions. Fractions that produce the same spot pattern were incorporated as a combined fraction of and tested for antifungal activity. The most active fraction was then analyzed by GC-MS to know the types of chemical compounds contained in this fraction.

RESULTS AND DISCUSSION

Inhibitory Activity of Partitioned Extract

Based on the results of partition using counter-current distribution method with two types of solvents are hexan and methanol phase showed that the methanol extract could inhibit the growth of *Colletotrichum acutatum* with the diameter of inhibition zone of 28 mm, whereas hexane extract phase could not inhibit the growth of this fungus (Figure 2). These results indicate that the active compounds in the leaf extract of *Mansoa alliacea* that are antifungal against *C. acutatum* is in the phase of methanol and is polar.

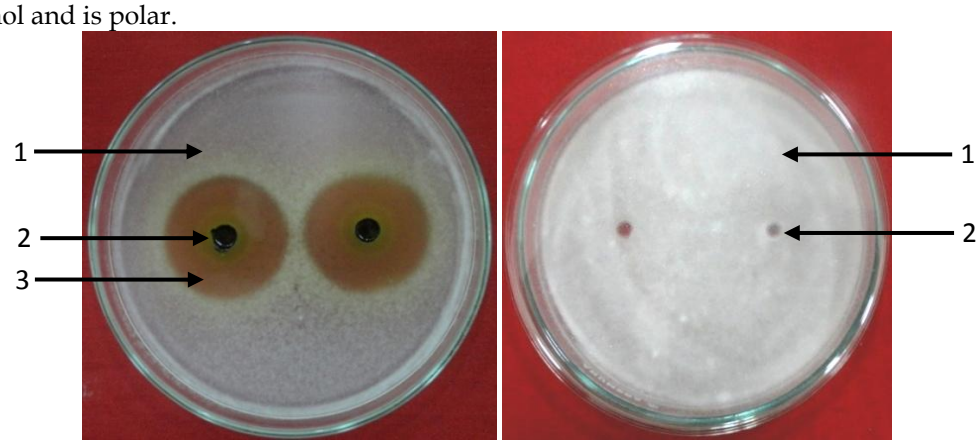

Figure 2. Photos of inhibition zone formed around the well diffusion filled with partitioned leaf extract of *Mansoa alliacea* of methanol phase (A) and hexane phase (B). (1 = mycelium of *C. acutatum*, 2 = well diffusion, and 3 = inhibition zone).

Gawade at al. (2014) reported the leaf extract of *Aegle Marmelos* (L). can inhibit the growth of the *Colletotrichum acutatum* with inhibition zone diameter of 22 mm. According to Patel et al. (2013) extract methanol of that leaves, roots and bark of *Mansoa alliacea* contain active compounds with the highest content of phenol (16.20 mg / g) found in leaves and flavonoids in roots (3.27 mg / g). But has not been any report on the bioactive compound of leaf extract from *Manosa alliacea* has a potential as botanical fungicides to control the anthracnose disease cause of *C. acutatum* on chili pepper. The size of the inhibition of a plant extracts against fungus varies greatly with type and concentration of compounds (Suprapta, 2001). Echegoyen et al. (2014) reported that alliin and the enzyme alliinase combine to produce allicin, a plant-defense chemical with a short half-life which they suggested to be responsible for the anti-microbial and anti-cancer properties in garlic.

Towne et.al (2015) *Mansoa alliacea* contains allicin compounds such as alyl sulpida and polysulfide which are anti-bacterial compounds.

Phytochemicals Compound Leaf Extract of *Mansoa alliacea*

The phytochemical test of the methanolic leaf extract of *Manosa alliacea* showed that leaf extract of *Mansoa alliacea* containing compounds such as terpenoids, alkaloids, flavonoids, and phenols (Table1). According to Patel (2013) leaves methanol extract of *Mansoa alliacea* contains phenolic compounds, alkaloids and flavonoids. Whereas according to Zoghbi et al. (2009) *Mansoa alliacea* leaf extract contains alkaloid compounds, flavonoids, phenols, steroids and terpenoids.

Table 1. Phytochemical test results of leaf extract of *Mansoa alliacea.*

Phytochemical test	Reaction result	Conclusion
Alkaloid	chocolatesediment	Alkaloid (+)
Triterpenoid	yellow to purple	Triterpenoid (+)
Phenolat	Blackish blue	Polyphenol (+)
Flavonoid	yellow	Flavonoid (+)
Saponin	Foamis not Constant	Saponin (-)
Tannin	No sediment is formed	Tannin (-)

Pires et al. (2017) melaporkan bahwa ekstrak kasar daun *Mansoa alliacea* mengandung senyawa fenolik dan flavonoid, jenis senyawa fenolik yang terkandung antara lain catechin, garllic acid, caffeic acid, ferllic acid dan quercitrin. Nduagu et al. (2008) reported the phytochemical content extracts of bark and root bark of plants can inhibit the growth of *Colletotrichum capsici* causes anthracnose in pepper. The extract positive for chemical compounds such as alkaloids (*Citrus limon* and *Azadirachta indica*), tannins (*Vernonia amygdalina*, *Azadirachta indica* and *Ocimum gratissimum*), glycosides (*Vernonia amygdalina*, *Citrus limon*, *Azadirachta indica*, *Ocimum gratissimum* and *Anona senegalensis*), saponins (*Vernonia amygdalina* , *Citrus limon*, *Azadirachta indica*, *Ocimum gratissimum* and *Anona senegalensis*) and flavonoids (*Azadirachta indica*).

Bioactive Compounds of Leaf Extract of *Mansoa alliacea* based on GC-MS Analysis

Phase active of leaf extract of *Mansoa alliacea* to inhibition the *Colletotrichum acutatum* were the analyzed components contained there in by using GC-MS (GCMS-QP2010 Ultra SHIMADZU). Chromatogram of the fraction analysis results showed 4 peaks as shown in Figure 3, so it is assumed that crude extract of *Mansoa alliacea* leaves may contain a maximum of 4 active compounds that are antifungal against *C. acutatum*. Each emerging peak was further identified by mass spectroscopy, so that each compound has a specific mass fragmentation pattern.

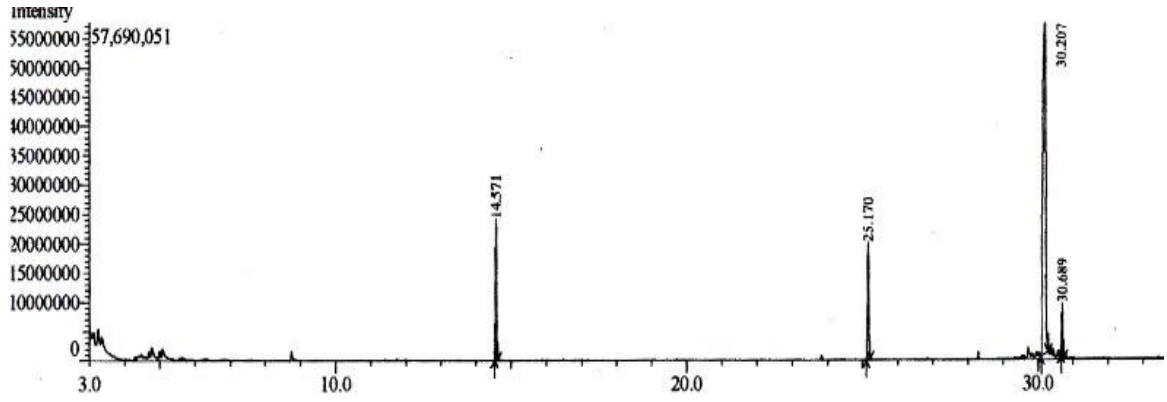

Figure 3. Chromatogram of GC-MS analysis of the active fractions of *Mansoa alliacea* capable of inhibiting the growth of *Colletotrichum acutatum*.

The identification was done by comparing the mass spectrum of each peak in the mass spectrum of compounds that are already known to exist in the GC-MS library.

Results of the analysis with GC-MS showed that the active phase of the leaf extract of *Mansoa alliacea* contains 4 compounds namely Pentan-1,3-dioldiisobutyrate,2,2,4; Tributyl acetylcitrate; Terephthalic acid, di(2-ethylhexyl) ester and 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all-E)-All-trans-Squalene. Three of them have been known as antifungal compounds. Those compounds are: Pentan-1,3-dioldiisobutyrate, 2,2,4; Tributyl acetylcitrate and Terephthalic acid, di(2-ethylhexyl) ester. The specification of each compound contained in the active phase of the leaf extract of *Mansoa alliacea* is presented in Table 2.

The spectrum of the peak spectrum in the mass of the GC-MS library. Results of analysis with GC-MS showed the active phase of the leaf extract of *Mansoa alliacea* contains 4 compounds namely Pentan-1,3-dioldiisobutyrate, 2,2,4; Tributyl acetylcitrate; Terephthalic acid, di (2-ethylhexyl) ester and 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl -, (all-E) -All-trans- Squalene. Three of them have been known as antifungal compounds. Those compounds are: Pentan-1,3-dioldiisobutyrate, 2,2,4; Tributyl acetylcitrate and Terephthalic acid, di (2-ethylhexyl) esters. The specification of the compound contained in the active phase of the extract extract from Mansa is presented in Table 2.

Table 2. Active compounds that have the potential as a botanical fungicide identified in the leaf extract of *Mansoa alliacea* based on the analysis with GC-MS.

No.	Peak	MW (molecular weight)	MF (molecular formula)	Retention time	Active coumpound base on database GC-MS
1.	Peak1	286	$C_{16}H_{30}O_4$	14.571	Pentan-1,3-dioldiisobutyrate, 2,2,4
2.	Peak2	402	$C_{20}H_{34}O_8$	25.170	Tributyl acetylcitrate
3.	Peak3	390	$C_{24}H_{38}O_4$	30.206	Terephthalic acid, di(2-ethylhexyl) ester
4.	Peak4	410	$C_{30}H_{50}$	30.689	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19, 23-hexamethyl-,(all-E)-All-trans-Squalence

According to Wright et al. (2017) *Tasmannia lanceolata* leaf extract from GC-MS analysis contains 2,2,4-trimethyl-1,3-pentanediol diisobutyrate which is a synonym of pentan-1,3-dioldiisobutyrate compound, 2,2,4-trimethyl, these compounds are antibacterial against *Clostridium perfringens*. Tanrattanakul and Bunkaew (2014) reported that the tributyl acetylcitrate compound extracted from *Adiatum capillus-veneris* leaves could inhibit bacterial and fungal growth including *Bacillus subtilis* bacteria, *Pseudomonas eurogenosa*, *Streptococcus faecalis*, *Salmonella typhi*, *Staphylococcus aureus* and *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus fumigates* and *Candida albicans*. According to Shobier et al. (2016) tributyl acetylcitrate compounds isolated from *Ulva lactuca* were able to inhibit the growth of *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus flavipes* and *Candida albicans*. Hameed et al. (2016) reported that the tributyl acetylcitrate compound isolated from *Cinnamomum zeylanicium* leaf extract could inhibit the growth of *Aspergillus flavus* and *Pseudomonas aerogenosa*, *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus* and *Klebsiella pneumonia*. According to Vivekrai et al. (2015) *Abutilon hirtum* leaf extract after being analyzed by GC-MS contained 19,17% terephthalic acid, di (2-ethylhexyl) ester compound which was used as a diuretic, antidiabetic, antibacterial and antifungal drug. Setianingrum (2014) reported that the compound 1,2-benzeneadicarboxylic acid, bis (2-ethylhexyl) ester is a fatty acid derivative compound that is antifungal against *Candida albicans*. According to de-Oliveira (2015) the 1,2-benzenedicarboxylic acid, 1,2-bis (2-ethylhexyl) ester compound isolated from the *Leonotis nepetifolia* has properties as an antifungal compound against *Candida albicans*, *Aspergillus* spp. and *Trichophyton* spp. Compound 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl has the name synonym squalene or trans-squalene or spinacen. Squalene is an intermediate or amphibolic compound in the process of metabolism.

According to Sudirga and Ginantra (2017) crude extract of *Ficus septica* leaves after being analyzed by GC-MS containing 14 active compounds including dl-glyceraldehyde dimer, 2,3,5 trimethyl heptane, Sulfurous acid cyclohexylmethylhexadecyl ester, guanosine, D-Allose, dodecanoic acid methyl ester, 1,2-Benzenedicarboxylic acid diethyl ester, 3-Deoxy-d-mannonic acid, cyclohexane tetraethyl 1,2,3,4, (Z) - 9-Tricosene, hexadecanoic acid methyl ester, octadecamethylcyclononasiloxane, 1-Heptacosanol and 1,2-Benzenedicarboxylic acid mono (2-ethylhexyl) esters and 8 of them are antifungal, 2,3,5 trimethyl heptane, Sulfurous acid cyclohexyl methylhexadecyl ester, dodecanoic acid methyl ester, 3-Deoxy-d-mannonic acid, hexadecanoic acid methyl ester, octadecamethylcyclononasiloxane, 1 - Heptacosanol and 1,2-Benzenedicar boxylic acid mono (2-ethylhexyl) ester.

CONCLUSION

The leaf extract of *Mansoa alliacea* contains several phytochemicals groups of compounds such as terpenoids, alkaloids, flavonoids, and phenols. The active phase of the leaf extract of *Mansoa alliacea* contains 4 compounds namely Pentan-1,3-dioldiisobutyrate,2,2,4; Tributyl acetylcitrate; Terephthalic acid, di(2-ethylhexyl) ester and 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all-E)-All-trans-Squalene. Three of them have been known as antifungal compounds. Those compounds are: 2 Pentan-1,3-dioldiisobutyrate,2,2,4; Tributyl acetylcitrate and Terephthalic acid, di(2-ethylhexyl) ester.

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