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Identification of the Substance Bioactive Leaf Extract *Piper caninum* Potential as Botanical Pesticides

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ABSTRACT

Based on the preliminary test of the 37 types of plants found that the crude extract of the leaves of P. caninum able to inhibit the growth of Pyricularia oryzae fungus the cause of rice blast disease on rice in vitro on PDA with inhibition zone diameter of 44 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. The method used is the method of column chromatography and thin layer, and GCMS. The study states that the extract of Piper caninum containing 4 secondary metabolites are alkaloids, folifenol, steroids and flavonoids and results analysis using GCMS there are 10 active compound is benzene; xylene; tetradecane; dodecanoic acid; heptadecane; hexadecanoic acid; octadecamethylocyclononasiloxane; phtalic acid; 8,11, 14-decosatrienoic acid; and 1,2-benzenedicarboxylic acid. Based on the existing references, of 10 compounds 6 compounds are: benzene; tetradecane; dodecanoic acid, hexadecanoic acid, benzenedicarboxylic acid, octadecamethylocyclononasiloxane, an active substance that serves as an antifungal and four other compounds unknown function.

Key words: *Piper caninum, extracts, bioactive substances, botanical pesticides*

INTRODUCTION

Indonesia is a country rich in biodiversity, either used as a source of food, medicine and as a botanical pesticide. Currently uses the plant as a pesticide plant developed, because it is one factor in supporting organic agriculture. The widespread use of synthetic pesticides causes environmental damage because it is not easily broken down in the environment. A total of 37,000 species of flora in Indonesia has potential as botanical pesticides. Semangun[11] found that an extract of the plant serves as a botanical pesticide if the plant extract can inhibit the development of a disease, because these plants contain bioactive substances such as flavonoids, polyphenols, alkaloids, saponins and tannins. Research Suriani *et al*[14], stated that the forest chilli leaf extract (*Piper caninum*) can inhibit the fungus *Pyricularia oryzae* causes rice blast disease.

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P. caninum is a creeper and climbing in a tree with a round stem, bark blackish green and hairy (Figure 1). *P. caninum* is found in tropical and subtropical regions, spreading from the lowlands to the highlands (1,100 asl). This plant likes height 600-800 meters above sea level[3].

Fig. 1: *Piper caninum* (Source: private collection, 2014)

According to Maj *et al*[7]., that *P. caninum* has an antimicrobial phytochemicals that act as antioxidants and as the 77.9 % found in the leaves and 87 % found in the trunk . This plant has anti- microbial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Pseudomonas putida, Escherichia coli*, *Candida albicans* , and *Aspergillus niger* . *All Piper contain phytochemical compounds evalonik acid types, cinanamoyl amides, alkhyl amides, aristolaktam,, flavones, dehidroflavone , dehidrochalcone , dehidroflavonoid*[7]. In addition, this plant extract can inhibit the growth of fungus because it contains 4, 5-dioxoaporphine alkaloid cepharadione A[7]. He said that *Piper* spp. (Piperaceae) contain bioactive substances include substances phenylpropanoids, lignoids, and plavonoids. Phenylpropanoids compounds act as insecticides, especially compounds dimethoxy -4,5- mthylenodioxy - allelbenzene (dilallpiol).

Crude extract of leaves of P. caninum able to inhibit the growth of *P. oryzae* fungus in vitro on PDA with inhibition zone diameter of 44 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.

MATERIALS AND METHODS

P. caninum leaves were collected from plants grown in the Village of Senganan, Penebel District, Tabanan Regency Bali. The leaves collected were mature leaves number 3 from top to bottom[5]. Collected leaves were washed with clean water to remove contaminants, then cut to small pieces and wind dried for 3 days in the shade. The materials were then macerated in methanol at a ratio of 1:10 (weight / volume) for 48 hours in the dark, at room temperature. The filtrate was obtained by filtering, using 4 layers of gauze followed by filtration using Whatman filter paper No. 1. The maceration process was done 3 times with methanol. The filtrate obtained were combined and then evaporated using a rotary evaporator (Iwaki, Tokyo) at 40° C to separate the solvent (methanol) and the extract. The crude extract obtained was ready for the next test.

Extract separation of methanol and hexane phases

Crude extract of *p. caninum* leaf as much as 4 ml was dissolved in 200 ml of methanol and 200 ml of hexane. The mixture was shaken in a separating funnel so that it was evenly mixed. This suspension was allowed to stand some time until visible phase separation between methanol and hexane phase. The two phases were separated and each phase with the solvent was evaporated in a rotary vacuum evaporator thus obtaining methanol and hexane extracts. Both extracts were tested for inhibitory against *P. oryzae* on PDA with the well diffusion method. Extracts showinggreater inhibition of activity were further fractionated by column chromatography.

Fractionation of active components

Extracts showed fungicidal activity against *P. oyzae* fractionated by column chromatography. Extract as much as 10 g dissolved in 40 ml of hexan. Once completely dissolved, add 10 g of silica gel 60 (0.063 to 0.200 mm) for column chromatography (70-230 mesh ASTM) Merck KGaA, 64271 Damstadt, Germany. The mixture was then evaporated to crumb using a rotary vacuum evaporator.

Crumbs extract on silica gel was put into chromatography column with the length of 59 cm and a diameter of 3.2 cm. The column had previously been charged with 115 grams of silica gel (Wako gel, particle size 75-150 m) and mixed in 350 ml of hexane. To get a fraction of the crude extract, the column was skipped with eluent (solvent) with the polarity of different levels, from the non-polar (hexane) followed by solvents that were more polar.

The eluent used in column chromatography in the order of polarity, from nonpolar to polar, in the following order:

1. Hexane, hexane: dichloromethane, dichloromethane, dichloromethane: ethyl acetate, ethyl acetate, ethyl acetate: acetone, Acetone, Acetone: propanol, propanol, propanol: ethanol, Ethanol, Ethanol: methanol, Methanol.
2. The eluate was collected each 50 ml in a bottle container. First as a bottle bin, the next bin as the second bottle and so on. Each eluate was evaporated. Then antifungal testwas done. The most active fractions were then used for the isolation and identification of chemical compounds.

Phytochemical test

Phytochemical test was performed to determine the compound[5] of the active fraction obtained, using a reagent of specific classes of compounds. Test for alkaloids, flavonoids, steroids, triterpenoids, phenol, saponins and tannins.

Analysis GC-MS (*Gas Chromatografhy-Mass Spectrofotometry*)

GC-MS analysis was conducted to identify active compounds which have antifungal activity against *P. oryzae* fungus, the cause of blast diseasein rice. Footage fractions which were most active and relatively pure were analyzed by GC-MS. Through the suitability of the molecular weight and fragmentation pattern of the isolated compounds with compounds in the library on GC-MS system, so that the isolated compounds were known of their structure. This test was conducted in the Joint Laboratory of the Faculty of Mathematics and Natural Sciences, Udayana Univerity.

RESULTS AND DISCUSSION

Inhibiting activities of partition result of forest chili leaf extract

Results partitions with counter current distribution method, using 2 types of solvents are hexan (non-polar) and methanol (polar) showed that the leaf extract of forest chili methanol phase showed inhibition against *P. oryzae* fungus and the hexan phase does not show any inhibition, Diameter zone barriers generated by the methanol extract phase is 28 mm. These results indicate that the bioactive compounds contained in extracts of *P. caninum* including polar compounds.

This is consistent with the statement Manjappa[6] that extracts of the ethanol and methanol *Chromoluena odorata L*. plants can inhibit the growth of fungal mycelia of *P.*

oryzae at a concentration of 2.5% by the inhibition of respectively 70.6% and 46.8%. Likewise Suprapta[13] states that methanol of the plant *Thymus vulgaris* extract can inhibit the growth of pathogenic fungus in tomato.

Results Fractionation Active Components

Fractionated results with column chromatography obtained two active fractions, namely fraction 16 and fraction 17 which showed inhibition against *P. oryzae* with a zone diameter of each inhibition of 10 mm and 30 mm. Furthermore, fraction 17 was active at TLC. The nicest results TLC was using dichloromethane: ethyl acetate 1: 1, obtained three stains or spots with each Rf value 0.93; 0.83, and 0.71.

The size of the inhibition of a plant extract against fungus varies greatly depending on the type and concentration of compounds[12]. *Cem-cem* leaf extract (*Spondias pinnata*) can inhibit the growth of bacteria *Erwinia chrysantheni*, the bacterium that causes soft rot in aloe vera, with 27.55 mm zone of inhibition, because it contains a condensed tannin potential as a botanical pesticides[1].

Phytochemical test

The test results showed that the forest chili leaf extract contains several phytochemicals groups of compounds such as alkaloids, steroids, polyphenol and flavonoids (Table1).

Table 1: Phytochemical test results of combined fraction

Phytochemicaltest	Reaction result	Conclusion
Alkaloid	chocolatesediment	Alkaloid (+)
Triterpenoid and Steroid	Chocolate	Steroid (+)
Phenolat	Blackish blue	Polyphenol (+)
Flavonoid	yellow	Flavonoid (+)
Saponin	Foamis not Constant	Saponin (-)
Tannin	No sediment is formed	Tannin (-)

Crude extract *Epicoccum sp*. can inhibit the growth and spore colonies of *M. oryzae* because they contain secondary metabolites of flavinine types. Secondary metabolites such as alkaloids and flavonoids are potential as antimicrobial and antifungal[4]. This statement is supported by Nadri *et al*[8]., that the methanol extract of *P. caninum* containing 108.43 phenolic compounds, ethyl acetate extract containing 51.73 phenolic compounds. Salleh *et al*[10]., reported *P. caninum* bark extract contains flavonoid compounds that have inhibitory effect on the microbial species *Escherichia coli*, *Staphylococcus aureus*, *Bacillus substilis*, *Pseudomonas aeroginosa*, and *P. putida* of 125-1000 mg / ml.

Identification results of active antifungal compounds by gas chromatography–mass spectroscopy (GC-MS).

Fractionation results with column chromatography results of which showed antifungal activity against *P. oryzae* were then analyzed using GC-MS. Chromatogram analysis results obtained showed 10 peaks (Figure 2), each peak was identified further by mass spectroscopy. Results of the analysis of the mass spectrum of the chromatogram are presented in Table 2.

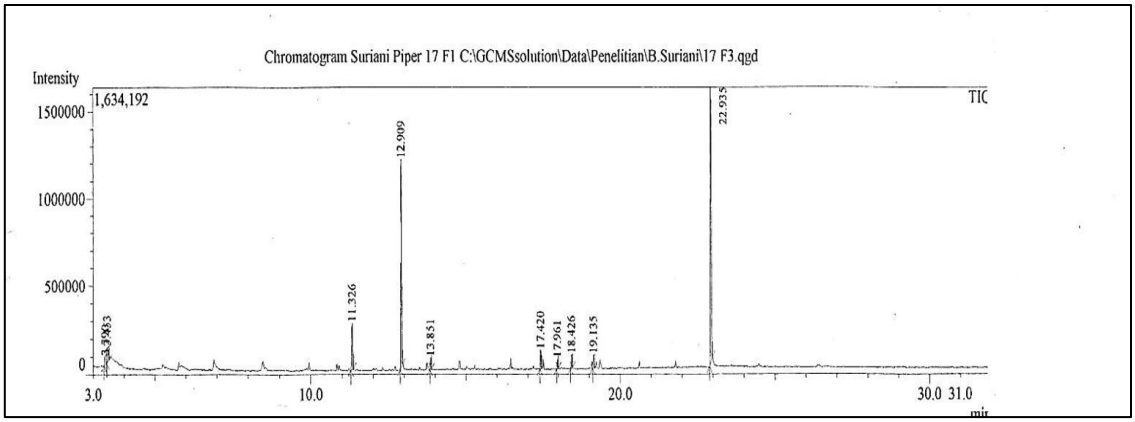

Fig. 2: Chromatogram compound in forest chili extract GS-MS analytical results

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. According to Appuaka *et al*[2]., the utility of the benzene compound is the most important thing as a solvent and as a raw material for making other aromatic compounds which are derivatives of benzene. These compounds act as antioxidants, antifungal and antimicrobial. Compound tetradecane hydrocarbon is used as a solvent and as a standard, as a raw material in chromatographic analysis. This material can cause lung damage if swallowed and vapors may cause drowsiness and dizziness. Tetradecane compounds including compounds act as antimicrobial and antifungal. Warsinah[15] reported that dodecanoic acid contained in the harp bark extract has biological activity as an antifungal. Dodecanoic acid is a fatty acid is generally known as lauric acid. Warsinah[15] reported that dodecanoic acid contained in the harp bark extract has biological activity as an antifungal. Dodecanoic acid is a fatty acid is generally known as lauric acid. 1,2-Benzenedicarboxylic acid ethyl ester is a compound that is commonly known as monoetilhexil phthalate. Raman *et al*[9]., reported that, 2-benzenedicarboxylic acid has biological activity as antimicrobial, antioxidant and anticancer. According to Appuaka *et al*[2]., that the hexane extract of leaves of neem (*Azadirachta indica*) containing 1.2 -benzenedicarboxylic acid compound is an anti-fungal compounds for the fungus *Candida albicans*, antibacterial for the bacterium *Salmonella typhi*.

Table 2: Active compounds from each of the peaks in the chromatogram of active fraction antifungal in forest chili leaf extract

No.	Peak	MW (molecule weight)	FM (Rms molecule)	Retention time (minute)	Area	Active compoundbased on MS database
1	Peak 1	106	C_8H_{10}	3,300	47688	Benzene
2	Peak 2	166	$C_{10}H_{14}O_2$	3,457	40974	Xylene
3	Peak 3	198	$C_{14}H_{30}$	11,32	111881	Tetradecane
4	Peak 4	214	$C_{13}H_{26}O_2$	12,909	454456	Dodecanoic acid
5	Peak 5	398	$C_{21}H_{14}$	13,851	24038	Heptadecane
6	Peak 6	270	$C_{17}H_{34}O2$	17,420	36460	Hexadecanoic acid
7	Peak 7	666	$C_{18}H_{54}O_9S_{19}$	17,963	12973	Octadecamethylcyclononasiloxane
8	Peak 8	292	$C_{17}H_{24}O_4$	18,426	34013	Phthalic acid
9	Peak 9	348	$C_{23}H_{40}O_2$	19,136	8641	8,11, 14-Docosatrienoic acid
10	Peak 10	278	$C_{16}H_{22}O_4$	22,935	82,531	1,2-Benzenedicarboxy1lic acid

CONCLUSION

The leaf extract of *P. caninum* contains several phytochemicals groups of compounds such as alkaloids, steroids, polyphenol and flavonoids. The compound contained in the active fraction of leaf extract of *P. caninum* as many as 10 compounds are: benzene; xylene; tetradecane; doecanoic acid; heptadecane; hexadecanoic acid; octadecamethylocyclononasiloxane; phtalic acid; 8,11, 14-decosatrienoic acid; and 1,2-benzenedicarboxylic acid. Out of the 10 compounds, the 6 compounds namely benzene; tetradecane; dodecanoic acid, hexadecanoic acid, benzenedicarboxylic acid, octadecamethylocyclononasiloxane, based on the literature, they are active substances that serve as antifungal and 4 other compounds have unknown functions.

Acknowledgement

Further studies are needed to separate the compounds present in the active fraction of forest chili leaves by using LC-MS because GC-MS uses the column temperature of 310^0C, so that compounds having a boiling point above 310^0 C are undetected. In addition, there should be further testing and refining to identify what compounds among the compounds found in the leaf extract play the greatest role in antifungal activity against *P. oryzae*.

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