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ORIGINAL ARTICLE

Antioxidant test of crude extract of secondary metabolites from *Fusarium* sp. isolate of rizosphere of cat's whisker

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Abstract

Antioxidants are compounds needed in the prevention and therapy of diseases such as diabetes mellitus, cancer and premature aging. Over time, secondary metabolite compounds as antioxidants can be obtained from microbes, namely those originating from the endophyte and rhizosphere of plants. Based on previous research, secondary metabolites that act as antioxidants are flavonoids and phenolics, with phenolic compounds and flavonoids can bind free radicals through hydroxyl groups present in their aromatic rings. Microbes that live on medicinal plants will have similarities in producing secondary metabolites which can also be used as medicine. *Fusarium* sp. Laboratorium Basic Science UNPRI (LBSU) isolate is an isolate isolated from the rhizosphere of the cat's whisker plant (*Orthosiphon stamineus*). The cat's whisker plant is known to be an antioxidant. This study aims to test the antioxidant crude extract of secondary metabolites from *Fusarium* sp. LBSU. The method for isolating secondary metabolites is by liquid fermentation, then extracted using ethyl acetate and tested for antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The antioxidant test results were obtained from the DPPH radical inhibition value by Ethyl acetate Extract of *Fusarium* sp. LBSU (EEF) is 44%, while Vitamin C is 88%. Thus, the ability to capture DPPH free radicals by EEF is 44%, weaker than Vitamin C.

Keywords: antioxidant, cat's whisker, fermentation, *Fusarium*, rizosphere

Introduction

Free radicals such as superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide and hydroxyl radicals ($HO^{\cdot}$) are highly reactive oxygen and are naturally produced as a result of cell metabolism in the body. High production of free radicals can lead to oxidative stress. This can stimulate cancer formation, inflammation, premature aging, atherosclerosis, and trigger diabetes mellitus.[1] In good condition, antioxidants can reduce the impact of free radical silencing and eliminate the harmful effects of oxidative stress.[2]

Antioxidants are bioactivities that are widely explored by researchers in the current era, as one of the therapeutics of diabetes mellitus, inhibitors of premature aging, anticancer and so on. The production of antioxidants from microbes has progressed a lot in research. Tu et al.[3] isolated *Fusarium oxysporum* for the production of antioxidant, cytotoxic and antimicrobial compounds. Marlinda et al.[4] also conducted antioxidant tests using secondary metabolites from *Fusarium oxyporum* LBKURCC41. In 2020[5], obtained positive results of antioxidant tests from fungi that had been isolated from the rhizosphere of *Phalaris arundinaceae* and *Scirpus syvaticus* plants. Murali et.al[6] reported that microbes that live around the roots and soil around the roots have a role in increasing the growth of their host plants, or called *plant growth promoting fungi* (PGPF).

PGPF can increase the growth of its host by helping nitrogen fixation, hormone production, *siderophore* production, producing hydrolysis enzymes, increasing antibiosis, and so on[6]. Based on a report from Murali et al., fungi that include PGPF are fungi with the genus *Trichoderma, Fusarium, Aspergillus* and

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Penicillium sp. In addition, *Fusarium oxysporum*, *Verticiliae dahlia*, *Phoma eupyrena*. *Fusarium* sp. has also been widely tested to improve the growth of lettuce, cucumber and spinach plants.[8-10]

PGPF can be isolated from plants used as medicine, such as cat's whisker (*Orthosiphon stamineus*). Cat whiskers are known as antihyperglycemia, antimicrobial, antioxidant, diuretic. Based on the results of research.[11] *O. stamineus* contains secondary metabolites including terpenoids, polyphenols, sterols, orthosyphols, saponins, flavonoids, caffeic acid, oleanoic acid, ursolic acid, betulinic acid, siphonol. *O. stamineus is* famous as a herbal in curing rheumatic diseases, diabetes, hypertension, epilepsy, gonorrhea, syphilis, influenza, and diseases that cause coronary heart disease such as hypercholesterolemia.[12] The content of phenolic compounds and flavonoids can bind free radicals through hydroxyl groups present in their aromatic rings.[13]

Fusarium sp. which is an isolate of the LBSU collection isolated from the roots of cat whisker plants. According to McNear[14], microbes that originate from the outermost layer of plant roots and are between extensive soil surfaces are called the *ectorhizosphere*. Based on the explanation above, *Fusarium* sp. LBSU will be utilized with the aim of producing antioxidant compounds, and aims specifically to produce secondary metabolite compounds as antioxidants derived from cat's whisker plant rhizosphere fungi. The urgency of this research is the utilization of rhizosphere fungi of cat whisker plants as antioxidants, namely from the genus *Fusarium* which has not been tested by previous researchers. The antioxidants obtained are expected to be used as active medicinal ingredients in the prevention of diseases such as diabetes mellitus and cancer. In this study, antioxidant test of secondary metabolites of *Fusarium* sp. LBSU will be conducted *in vitro*.

Method

The equipment used in this study were 1000 mL Erlenmeyer (Pyrex), test tube (IWAKI), 500 mL glass beaker (Pyrex), ose needle, FT-IR (Shimadzu), *shaker incubator (*Labnet), *rotary evaporator* (IKA), autoclave (Hirayama), separatory funnel (Duran), filter funnel and vacuum device, *blue tip, yellow tip*, micro pipette (Biorad), *Bioscience Safety Cabinet (*BSC), Smartreader (Accuris), drop pipette, and other glassware. The materials used in this study were rhizosphere isolates of *Fusarium* sp. LBSU, *Potato Dextrose Agar* (PDA) (Merck), *Potato Dextrose Broth (*PDB) (Merck), ethyl acetate, Sodium Chloride (Widatra Bhakti), distilled water, filter paper (Whatman No. 41, 125 mm), tissue, and other materials appropriate to the study. This research was conducted at the Basic Science Laboratory of Prima Indonesia University.

Culturization and production of secondary metabolites

Fusarium sp. LBSU was rejuvenated on PDA media in test tubes for 3-4 days at 30°C. After that, 3 ml of sterile 0.8% physiological NaCl solution was added to the culture tube and the suspension formed was pipetted as much as 0.1 ml to be inoculated onto PDA media in a Petri dish. The dish was incubated for 3-4 days at 30°C.[15] After the culture grew, the culture was taken using a *cork borer* with a diameter of 6 mm. Then 3 pieces of isolate were put into each 1 L Erlenmeyer containing 300 ml of sterile PDB media solution for the fermentation process. Erlenmeyer was incubated at 30°C in a *shaker incubator* for 3 weeks.[16]

After 3 weeks of incubation, the culture was filtered to separate the spores and mycelium formed using filter paper in a Buchner funnel with the help of a vacuum device. The filtrate was collected and secondary metabolites were extracted by mixing the filtrate with ethyl acetate with a volume equation of 1:1 to the total volume of filtrate. This extraction treatment was carried out twice. Filtrate and each solvent were mixed in a separatory funnel and allowed to stand for 1 hour, then shaken until homogeneous. After that, the mixture was allowed to stand until two phases were formed, namely the media water phase and the ethyl acetate phase. The phase above was collected to be evaporated in a *rotary evaporator* to obtain a concentrated crude extract.[17,18]

Antioxidant activity test

In this antioxidant test, the DPPH inhibitory activity method was used, where DPPH solution was prepared with a concentration of 0.1 M in methanol. The concentrated crude extract from ethyl acetate solvent was used in this antioxidant test in suspension form. The sample used was EEF (*Fusarium* ethyl ace-

Table 1. Compotition of formula antioxidant test of EEF, positive control, and negative control

Compotition of formula			
Sample Volume	**Methanol Volume**	**DPPH Volume**	
EEF 50 µl	200 µl	100 µl	EEF Sample
Vitamin C 50 µl	200 µl	100 µl	Positive control
		350 µl	Negative control

tate extract) and the positive control used was Vitamin C 50 mg with a concentration of 1000 ppm in distilled water. The formula used in this test can be seen in Table 1. The mixtures were stirred until homogeneous in a 96 well microplate and incubated at room temperature for 30 minutes in the dark. After completion of the incubation period, the microplate was inserted into a Smartreader to read the absorbance of the test results using a wavelength of 517 nm. Ascorbic acid was used as standard.[17] The antioxidant activity of EEF was measured as percentage using the formula: *scavenging activity (%)= A control- A sample / A control*.[18]

Results

Antioxidants can be obtained from natural resources such as plants, which have been used as herbal medicines. Not only that, with the development of technology and knowledge to date, antioxidants can be obtained from microbes such as fungi and bacteria, both in the form of endophytes and rhizospheres. The antioxidant test results in this study are crude extracts of secondary metabolites from rhizosphere isolates of *Fusarium* sp. with the code LBSU. *Fusarium* sp. LBSU used in this fermentation was incubated for 4 days (Figure 1). The crude extract was extracted using ethyl acetate solvent 1:1. Data on the percentage of DPPH free radical inhibition of EEF and Vitamin C can be seen in Table 2.

Figure 1. Top view of the rhizosphere isolate *Fusarium* sp. LBSU on PDA media in a petri dish incubated at 30°C for 4 days

Discussion

Antioxidants in this study were obtained from ethyl acetate crude extract of *Fusarium* sp. LBSU isolate, through liquid fermentation process (Figure 2). The liquid fermentation medium used was PDB media. PDB media is a basic media, containing dextrose, as well as carbohydrates and minerals sourced from potatoes. Dextrose is a monohydrate type carbohydrate, so there is no fungal activity to hydrolyze the carbon source into a single one. PDA and PDB are common media containing simple carbohydrates for good fungal growth.[19]

Fermentation incubation was carried out for 21 days, which is sufficient time for the fungus to produce secondary metabolites in the death phase. Abdullah[19] conducted liquid fermentation of endophytic fungus DGG-03 for 21 days, with the aim that the number of living fungal cells is balanced with the number of dead fungal cells. Supported by a statement from Rendowaty et al.[20], 21 days of mushroom fermentation incubation is an incubation period in which the growth of living fungal cells is equivalent to the number of non-living fungal cells, by utilizing nutrients derived from dead fungal cells (lysis).

Figure 2. Rhizosphere isolates of *Fusarium* sp. LBSU fermented in a *shaker incubator* for 21 days at 30°C

The secondary metabolite extract used in the study was obtained from ethyl acetate solvent which is semipolar (Figure 3). Semipolar is a solvent that can bind compounds that are nonpolar and polar, so that the extracted compounds can be between polar and nonpolar. According to Putri et al.[21], ethyl acetate is a solvent that has low toxicity, easily evaporated (volatile) at a temperature of 58-70 ° C, making it easier to extract and dry the concentrate extract obtained. Asmah et al.[22] stated that ethyl acetate has the highest dielectric constant of 6.02, compared to n-hexane (1.89) and chloroform (4.8). Oktiansyah et al.[23] conducted antioxi-

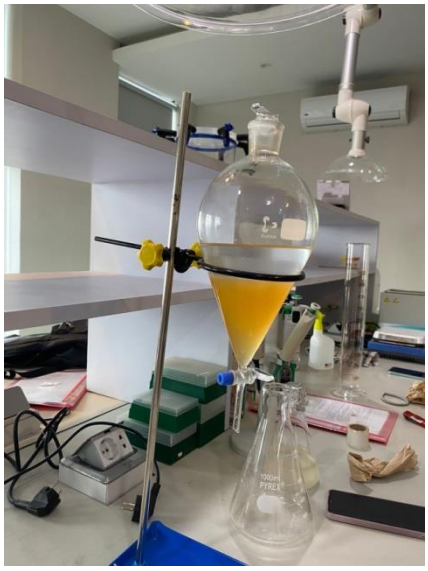

Figure 3. The extraction process of secondary metabolites from liquid fermentation media of rhizosphere isolates of *Fusarium* sp. LBSU

Table 2. Percentage of DPPH free radical inhibition of EEF and Vitamin C

Sample	Percentage of DPPH radical inhibition
EEF	44%
Vitamin C	88%

dant tests of ethyl acetate extract of *Trichoderma harzianum* endophyte isolate with IC50 radical inhibition power of 18.74 µg/ml. Hafsan[24] also used ethyl acetate to extract secondary metabolites from fungal endophytic isolates isolated from *Caesalpinia sappan*, namely *Trichoderma* sp. and *Penicillium* sp.

The inhibition value of DPPH radical by EEF was lower than 50%, which was 44%. The group of compounds that have activity as antioxidants are flavonoids and phenols. Phenols and flavonoids have a structure that can bind free radicals so that they have potential as antioxidants.[25] Marlinda[4] conducted an antioxidant test of ethyl acetate extract of *Fusarium oxysporum* endophytic isolate LBKURCC41, and had a low IC50 value of >250 µg/ml. Meanwhile[26], conducted research, namely antioxidant testing of ethyl acetate extract of *Fusarium oxysporum* which obtained a radical inhibition of 159.94 ppm. Compared to the DPPH radical inhibition of Vitamin C in this study which is 88%, the DPPH radical inhibition by EEF is low.

Conclusion

The antioxidant activity of EEF is 40%, which is lower than the antioxidant activity of Vitamin C which is 80%. This antioxidant activity can be improved in the future by using other solvents in order to extract other active compounds from rhizosphere isolates of *Fusarium* sp. LBSU.

References

1. Pizzino G, *et.al*. Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*. 2017. https://doi.org/10.1155/2017/8416763
2. Neha K, Haider MR, Pathak A, Yar MS. Medicinal prospects of antioxidants: A review. European Journal of Medicinal Chemistry. 2019;178: 687-704. https://doi.org/10.1016/j.ejmech.2019.06.010
3. Tu NHK, Thuy NTT, Vinh DTT, Anh NPQ, Ly HD. Detection of antimicrobial, antioxidant and cytotoxicity activities of *Fusarium oxysporum* F01 isolated from *catharanthus roseus* collected in Vietnam. J Pure Appl Microbiol. 2021;15(3): 1643-1654. doi: 10.22207/JPAM.15.3.61
4. Marlinda S, Teruna HY, Pratiwi NW, Ardhi A, Saryono. Antioxidants from extracts of the endophytic fungus *Fusarium oxysporum* LBKURCC41. Journal of Natur Indonesia. 2019;17(2): 1-9.
5. Hu S, Hu B, Chen Z, Vosatka M, Vymazal J. Antioxidant response in arbuscular mycorrhizal fungi inoculated wetland plants under Cr stress. Environmental Research. 2020. https://doi.org/10.1016/j.envres.2020.110203
6. Murali M, *et.al*. Bioprospecting of rhizosphere-resident fungi: their role and importance in sustainable agriculture. Journal of Fungi. 2021;7: 1-26. https://doi.org/10.3390/jof7040314
7. Xu L, Ravnskov S, Larsen J, Nicolaisen M. Linking fungal communities in roots, rhizosphere, and soil to the health status of *Pisum sativum*. FEMS Microbiol Ecol. 2012;82: 736-745. DOI: 10.1111/j.1574-6941.2012.01445.x
8. Minerdi D, Bossi S, Maffei ME, Gullino ML, Garibaldi A. *Fusarium oxysporum* and its bacterial consortium promote lettuce growth and expansin A5 gene expression through microbial volatile organic compound (MVOC) emission. FEMS Microbiol. Ecol. 2011;76, 342-351.
9. Saldajeno MGB, Hyakumachi M. The plant growth-promoting fungus *Fusarium equiseti* and the arbuscular mycorrhizal fungus *Glomus mosseae* stimulate plant growth and reduce the severity of anthracnose and damping off diseases in cucumber (*Cucumis sativus*) seedlings. Ann. Appl. Biol. 2011;159, 28-40.
10. Horinouchi H, Muslim A, Hyakumachi M. Biocontrol of *Fusarium* wilt of spinach by the plant growth promoting fungus *Fusarium equiseti* GF183. J. Plant Pathol. 2010;92, 249-254.
11. Jo YS, *et.al*. *Orthosiphon stamineus* Reduces Appetite and Visceral Fat in Rats. J. Korean Soc. Apl. Biol. Chem. 2011;54(2): 200-205.
12. Ameer, *et.al*. Orthosiphon stamineus: Traditional Uses, Phytochemistry, Pharmacology, and Toxicology. Journal of Medicinal Food. 2012;15(8): 678-690
13. Putri RDW, Nuniek H. Potential antioxidant compounds produced by endophytic bacteria in guava leaves (*Psidium guajava* L.). Journal of Chemistry. 2021;10 (1), 55-63.
14. McNear, D. H. 2017. The Rhizosphere - Roots, Soil and Everything In Between. *Nature Education*.

15. Piska F, Teruna HY, Saryono. Terpenoids as antibacterial produced by endophyte *Fusarium oxysporum* LBKURCC41 from *Dahlia variabilis* Tuber. J. Phys. Conf. Ser. 1655012034. 2020. DOI: 10.1088/1742-6596/1655/1/012034.
16. Sutejo AM, Priyatmojo, Wibowo A. Morphological identification of several species of *Fusarium* fungi. Indonesian Journal of Plant Protection. 2008;14(1): 7-13.
17. Habisukan UH, Elfita, Widjajanti H, Setiawan A. Secondary metabolite and antioxidant activity of endophytic fungi isolated from *Syzygium aqueum* leaves stalk. Biointerface Research in Applied Chemistry. 2022;12(6): 7584-7595. https://doi.org/10.33263/BRIAC126.75847595
18. Almanaa TN, Rabie G, El-Mekkawy RM, Yassin MA, Saleh N, El-Gazzar N. Antioxidant, antimicrobial and antiproliferative activities of fungal metabolites produced by *Aspergillus flavus* on *in vitro* study. Food Science and Technology. 2021. DOI: https://doi.org/10.1590/fst.01421.
19. Abdullah M, Fitriana, Maryam S. Antioxidant activity test of endophytic fungal isolates of galing-galing leaves (*Cayratia trifolia* l.) by 1,1-diphenyl-2-picrylhydrazil (DPPH) method. As-syifaa Journal of Pharmacy. 2020;12(2): 117-122.
20. Rendowaty A, Djamaan A, Handayani D. Optimal Cultivation Time and Antibacterial Activity of Ethyl Acetate Extract of Symbiont *Fungus Aspergillus unguis* (WR8) with *Haliclona fascigera*. Journal of Pharmaceutical Science. 2017;4(2):49-54.
21. Putri. WS, Warditiani NK, Larasanty LPF. Phytochemical screening of ethyl acetate extract of mangosteen fruit peel (*Garcinia mangostana* L.). 2013.
22. Asmah N, Dewi FS, Anggraini M, Alim ZM, Endang WB. Identification of active compounds in ethyl acetate, chloroform, and N-hexane extracts from peels of *Citrus aurantifolia* from Maribaya, West Java, Indonesia. J. Adv Pharm Technol Res. 2020;11(3): 107-112. doi: 10.4103/japtr.JAPTR_177_19
23. Oktiansyah R, Hapida Y, Risasti S. Antioxidant activity of endophytic fungi extract isolated from peel of pineapple (*Ananas comosus* (L.) Merr. 'Prabumulih')). Scientific Journal of Mathematics and Natural Sciences. 2023;20(2): 114-125. DOI 10.31851/sainmatika.v20i2.13270
24. Hafsan H, Sukmawaty E, Masri M, Aziz IR, Wulandari SL. Antioxidant activities of ethyl acetic extract of endophytic fungi from *Caesalpinia sappan* L. and *Eucheuma* sp. International Journal of Pharmaceutical Research. 2018;10(3): 239-244.
25. Hikmah N, Enos TA, Sukemi. Phenolic and flavonoid compounds, and antioxidant activity of methanol extract of ihau (*Dimocarpus longan* Lour var. malesianus Leenh.) fruit peel. Bivalent: Chemical Studies Journal. 2020;3(2): 39-42.
26. Purbaya S, Gina F, Lutfia SA, *et.al.* Antioxidant activity of compound 6-propyl-5,*6-dihydro-2H-piran-2-on* from endophytic fungus *Fusarium oxysporum Kalanchoe millotii* root. J. Kartika Kimia. 2023;6(2): 109-115. DOI: https://doi.org/10.26874/jkk.v6i2.211