PROCEEDING

The 2nd International Seminar of Basic Science

Natural Science For Exploration The Sea-Island Resources

Ambon, May 31st 2016

Organized by

Faculty of Mathematics and Natural Science

Pattimura University

PROCEEDINGS

The 2nd International Seminar of Basic Science

"Natural Science for Exploration The Sea-Island Resources"

Poka-Ambon, 31st May 2016

Mathematic and Natural Science Faculty
Universitas Pattimura
Ambon
2016

PROCEEDINGS

The 2nd International Seminar of Basic Science
May, 31st 2016

ISBN : 978-602-97522-2-9

Mathematic and Natural Science Faculty
Universitas Pattimura
Ir. M. Putuhena St.
Kampus Poka-Ambon
Pos Code 97233
Email:fmipa_unpatti@gmail.com

2nd edition

Welcoming Address By The Organizing Committee

Today, We have to thank the The Almighty Allah SWT for the implementation of this international seminar. This is the second seminar about Basic Science in The Faculty of MIPA Pattimura University. The seminar under the title “Natural Sciences for Exploration the Sea-Island Resources” will be carried out on May 31st 2016 at Rectorate Building, Pattimura University. There are 200 participants from lecturers, research institute, students, and also there are 34 papers will be presented.

My special thanks refer to the rector of Pattimura University and the Dean of MIPA Faculty, Prof. Dr. Pieter Kakissina, S.Pd., M.Si. I also would like to express my deepest gratitude to Prof. Amanda Reichelt-Brushett, M.Sc., Ph.D. ; Kazuhiko Ishikawa, Ph.D. ; Nicolas Hubert, Ph.D. ; Prof. Dr. Kirbani Sri Brotopuspito ; Prof. Dr. Marjono, M.Phil. ; Gino V. Limon, M.Sc., Ph.D. as the keynote speakers.

The last, We hope this international seminar usefull for all of us, especially Mollucas People and very sorry if any mistake. Thank you very much.

Dr. La Eddy, M.Si.
Chairman of Organizing Committee

Opening Remarks
By Dean of Mathematic and Natural Sciences Faculty

I express my deepest gratitude to The Almighty God for every single blessing He provides us especially in the process of holding the seminar until publishing the proceeding of International Seminar in celebrating the 18th anniversary of MIPA Faculty, Pattimura University. The theme of the anniversary is under the title "Natural Sciences for Exploration the Sea-Island Resources". The reason of choosing this theme is that Maluku is one of five areas in Techno Park Marine in Indonesia. Furthermore, it is expected that this development can be means where the process of innovation, it is the conversion of science and technology into economic value can be worthwhile for public welfare especially coastal communities.

Having the second big variety of biological resources in the world, Indonesia is rich of its marine flora and fauna. These potential resources can be treated as high value products that demand by international market. Basic science of MIPA plays important role in developing the management of sustainable marine biological resources.

The scientific articles in this proceeding are the results of research and they are analyzed scientifically. It is expected that this proceeding can be valuable information in terms of developing science and technology for public welfare, especially people in Maluku.

My special thanks refer to all researchers and reviewers for your brilliant ideas in completing and publishing this proceeding. I also would like to express my gratefulness to the dies committee-anniversary of MIPA Faculty for your creativity and hard working in finishing this proceeding, God Bless you all.

Prof. Dr. Pieter Kakisina, S.Pd., M.Si.
Dean of Mathematic and Natural Sciences Faculty

ACKNOWLEDGMENT

The following personal and organization are greatfully

acknowledgment for supporting

"The 2nd International Seminar of Basic Science 2016"

Hotel Mutiara Ambon

Contents

ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF ENDOPHYTIC FUNGI FROM SIRIH HITAM PLANT (*Piper* betel L)

Edward J. Dompeipen

Institute of Research and Standardization Industry of Ambon
Kebun Cengkeh Street, Ambon, Moluccas, Indonesia
Email: dompeipenedward@yahoo.com

ABSTRACT

Diabetes Mellitus is a degenerative disease characterized by hyperglycemia due to the bodies absolute insulin and relative insulin deficiency. The study was aimed to isolate endophytic fungi from stem of Sirih hitam (Piper betel L) plant was active as antidiabetic and antioxidant. Antidiabetic activity conducted by using the α-glucosidase inhibitory and antioxidant activity using free radical reduction method with reagent 2.2-diphenyl-1-picrylhydrazyl (DPPH). Isolation of microbes conducted in the media Corn Meal Malt Agar (CMMA) and Potato Dextrose Agar (PDA) and obtained 9 isolates of fungus.Inhibitory activity against α-glucosidase to extract the filtrate and biomass of the isolates A.Ps.1F (55.45 and 80.22%), A.Ps.4F(57.40 and 51.48%), A.Ps.5F (70.92 and 70.92%), and B.Ps.1F (62.23 and 53.51%).) thus has potentials an antidiabetic activity. A.Ps.1F were the only isolates active as antioxidants with IC_{50} of 95.09 ppm.

***Keywords*:** Piper betel L, α -glucosidase, 2,2-difenil-1-pikrilhidrazil, antidiabetic,antioxidant

INTRODUCTION

The Piper betel plant is an evergreen and perennial creeper which is used in several traditional medicines to cure various diseases. This plant has been known to possess antioxidant, antifungal, antiulcerogenic, antiplatelet, antidiabetic, immunomodulatory, antileishmanial, antiamoebic, anti-inflammatory, antifilarial and antimicrobial activity. A wide range of chemical compounds including chavibetol, allyl pyrocatechol, eugenol, quercetin, caryophyllene, safrole, hydroxychavicol, a-pinene, myrcene, chavicol, germacrene-D, a-terpineol , ß-pinene, camphene etc have been isolated from this plant (Kushagra Nagory, et al, 2016)**.**

The term “endophytes” includes a suite of microorganisms that grow intra-and/or intercelullarly in the tissues of higher plants without causing over symptoms on the plants in which they live, and have proven to be rich sources of bioactive natural products (Li., et.al. 2008). Mutualism interaction between endophytes and host plants may result in fitness benefits for both partners (Kogel.et.al.,2006). The endophytes may provide protection and survival conditions to their host plant by producing a plethora of substances which, once isolated and characterized, may also have potential for use in industry, agriculture, and medicine (Stroble.,et.al.2004).

The production of bioactive substances by endophytes is directly related to the independent evolution of these microorganisms, which may have incorporated genetic information from higher plants, allowing them to better adapt to plant host and carry out some functions such as protection from pathogens, insects, and grazing animals (Strobel, G.A, 2003). Endophytes are chemical synthesizer inside plants (N. L. Owen and N. Hundley, 2004), in other words, they play a role as a selection system for microbes to produce bioactive substances with low toxicity toward higher organisms (Strobel, G.A, 2003).

Bioactive natural compounds produced by endophytes have been promising potential usefulness in safety and human health concerns, although there is still a significant demand of drug industry for synthetic products due to economic and time-consuming reasons (Strobel and Daisy, 2003). Problems related to human health such as the development of drug resistance in human pathogenic bacteria, fungal infections, and life threatening virus claim for new therapeutic agents for effective treatment of diseases in human, plants, and animals that are currently unmet (Zhang, et al, 2005).

Recent review by Newman and Cragg (D. J. Newman and G. M. Cragg, 2007), from which a significant number of natural drugs are produced by microbes and/or endophytes. Endophytes provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, and others (R. X. Tan and W. X. Zou). Such bioactive metabolites find wide-ranging application as agrochemicals, antibiotics, immunosuppressants, antiparasitics, antioxidants, antidiabetic and anticancer agents (Gunatilaka, 2006).

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. Recently compiled data show that approximately 150 million people have diabetes mellitus worldwide, and that this number may well double by the year 2025. Much of this increase will occur in developing countries and will be due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles. By 2025, while most people with diabetes in developed countries will be aged 65 years or more, in developing countries most will be in the 45-64 year age bracket and affected in their most productive years (WHO Media Centre, 2016).

Complications of diabetes associated with oxidative stress in particular the formation of superoxide free radicals (Oberley, et.al., 1998; Erawaty, 2012). Sources of oxidative stress in diabetes mellitus include the transfer of the balance of redox reaction due to differences in carbohydrate and lipid metabolism which will increase the formation of ROS (Reactive Oxygen Species) of the results of glycation and oxidation of lipids thus lowering the antioxidant defense system including glutathione (GSH) (Halliwell et. al., 1997; Erawaty, 2012). Hyperglycemia will aggravate and exacerbate the formation of ROS through several mechanisms (Tiwary, et.al., 2002).

The study was aimed to isolate endophytic fungi from Sirih hitam (Piper betel L) plant stem was active as antidiabetic and antioxidant. Antidiabetic activity conducted by using the –α glucosidase inhibitory and antioxidant activity using free radical reduction method with reagent 2.2-diphenyl-1-picrylhydrazyl (DPPH).

MATERIALS AND METHODS

Material

Research materials are species of well known Indonesian medicinal plants for antidiabetic: Sirih hitam (Piper betel L) collected from Bogor.

Isolation and purification of endophytic microbes

Plant's stem in 2 cm for length and 1 cm for diameter were washed by water followed by sterilization using ethanol 70% for 1 min and ethanol 75% for 30 sec. Stems were cut longitudinally into 2 pieces then were placed on petri disc filled with Corn Meal Malt Agar (CMMA) mixed with 0.05 mg mL-1 chloramphenicol (Theantana et al., 2007). Others were placed on Potato Dextrose Agar (PDA) mixed with 0.05 mg mL-1 chloramphenicol (Croizer et al., 2006). Those agars were incubated at 25°C for 3 days. Purification were done by transferring colony to fresh PDA followed by incubation at 25°C for few times until we got pure colony. Pure colony are kept on slanted PDA in the refrigerator (-80°C) for further treatment.

Production of endophytic microbes bioactive compounds

Production and fermentation of endophytic microbes chemical compounds were done by cutting 1 week old endophytic microbe colony in medium agar using cork borer tobe inoculum disc with diameter of 1.8 cm. Inoculum was inoculated into 250 Potato Dextrose Broth (PDB) medium in Erlenmeyer flask size 1000 mL and fermented for 14 days with agitation 150 rpm at room temperature (Onifade, 2007). Fermented fungi were extracted using ethylacetate and filtrated using Whatman filter paper number 41 until we got filtrate and biomass of fungi. Filtrate collected was evaporated using evaporator and were dried using oven at 40°C. Dried biomass was extracted again using ethylacetate to get the extract (Sunil et al., 2009).

In vitro antidiabetic activity test of active compounds

Screening was done to get isolates which can produce a chemical compound with inhibitory activity to α-glucosidase enzyme. In this screening, we were having test to all ethylacetate extracts of endophytic fungi (both filtrate and biomass extracts). As control we used quercetin, a well known flavonoid compound which have inhibitory activity to α-glucosidase enzyme. All samples and standard solution were measured at concentration of 50 ppm (Suarsana et al., 2008).

In vitro antidiabetic activity test were done using α-glucosidase method (Saijyo et al., 2008). Inhibition capability were calculated using formula:

$$\boldsymbol{Inhibition}\ (\%) = \frac{\boldsymbol{C}}{\boldsymbol{S}} \times \mathbf{100}$$

where, S showed sample absorbance and C showed blank absorbance with quercetin solution as standard.

Antioxidant activity test

Antioxidant activity test using scavenging free radicals by using reagent DPPH (2,2 - diphenyl-1- picryl hydrazyl) (Gurav, et.al., 2007;Erawati, 2012).

Thin layer chromatography test

Both filtrate and biomass ethylacetate extract of endophyte isolates were tested by thin layer chromatography analysis using eluent chloroform-methanol (5:1) and silica gel GF_{254} as solid phase. Spray reagent used is serium sulphate.

RESULTS AND DISCUSSION

Isolation of endophytic fungi

Isolation and purification of endophytic fungi from Sirih hitam (*Piper betel* L.) gave results 9 fungi isolates. Complete results of isolation can be seen in Table 1.

Table 1. Endophytic fungi isolated from stem of Piper betel plant

No	Plant	Fungi Isolate	Ethylacetate Extract Weight	
			Filtrate (g)	Biomass (g)
1	Piper betel L	A.Ps.1F	0,0133	0,0100
2	Piper betel L	A.Ps.2F	0,0089	0,0085
3	Piper betel L	A.Ps.3F	0,0120	0,0879
4	Piper betel L	A.Ps.4F	0,0064	0,0143
5	Piper betel L	A.Ps.5F	0,0045	0,0184
6	Piper betel L	B.Ps.1F	0,0113	0,0226
7	Piper betel L	B.Ps.2F	0,0065	0,0115
8	Piper betel L	B.Ps.3F	0,0148	0,0119
9	Piper betel L	B.Ps.4F	0,0063	0,0157

Notation A is refers to endophytic fungi isolates planted on CMM (Corn Meal Medium), Notation B is refers to endophytic fungi isolates planted on PDA (Potato Dextrose Agar)

Research conducted by Anuree Khanbun in Thailand managed to isolate the 22 endophytic fungi on the leaves of the Piper betel in PDA media. Endophytic isolates were then tested to produce antimicrobial compounds. Endophytic fungi isolates PBL 004 can produce compounds that have antimicrobial activity against Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231 and Escherichia coli ATCC 25 922 (Anuree Khanbun, 2004).

.

Figure isolate endophytic fungi from stem of Piper betel Plant

Isolates of Endophytic fungi from stem of Piper betel plant can be seen in Figure 1, after shooting microscopically at 4000 times magnification.

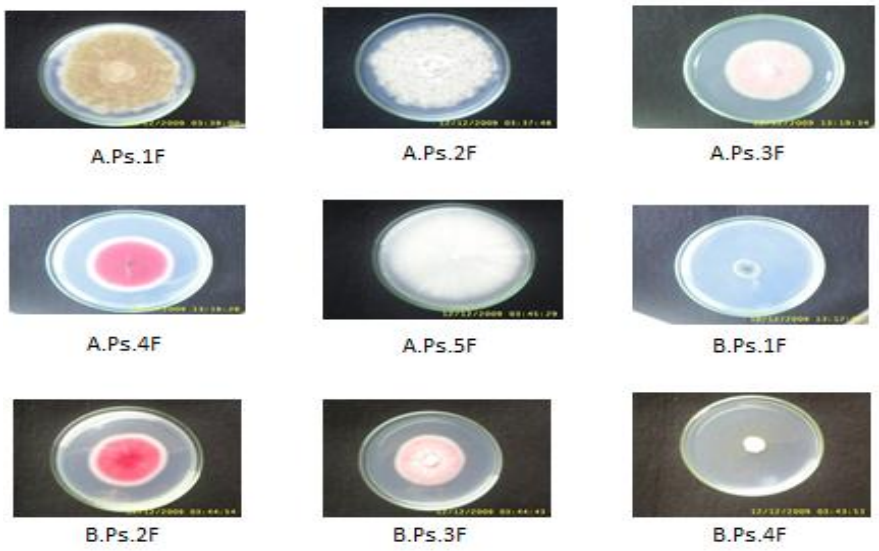

Figure 1. Isolates endophytic fungi from stem of Piper betel plant

Screening of endophytic fungi isolates using α-glucosidase test:

α-glucosidase is an enzyme in intestine which can hydrolyze carbohydrate into simple sugar (glucose). Compound which can inhibit this enzyme activity will be very potential to be an antidiabetic drug since it can decrease blood sugar level by slowing the absorption of carbohydrate *post-prandial*. The results of the screening were summarized in Table 2.

Table 2. The results of the screening of endophytic fungi isolates using α-glucosidase test

No	Plant	Fungi Isolate	Absorbance		α-Glucosidase (100%)	
			Filtrate	Biomass	Filtrate	Biomass
1	Piper betel L	A.Ps.1F	0,0527	0,0234	55,45	80,22
2	Piper betel L	A.Ps.2F	0,0842	0,0344	28,83	70,92
3	Piper betel L	A.Ps.3F	0,0300	0,0892	74,64	24,60
4	Piper betel L	A.Ps.4F	0,0504	0,0574	57,40	51,48
5	Piper betel L	A.Ps.5F	0,0344	0,0344	70,92	70,92
6	Piper betel L	B.Ps.1F	0,0435	0,0550	62,23	53,51
7	Piper betel L	B.Ps.2F	0,0344	0,0598	70,92	49,45
8	Piper betel L	B.Ps.3F	0,0598	0,0458	49,45	61,28
9	Piper betel L	B.Ps.4F	0,0621	0,0278	47,51	76,50
10	Standard	Acarbose			98.28	

In this test, we compared absorbancy of standard with samples. DMSO solution was used as blank solution while quercetin as standard. Absorbancy value of DMSO solution 0.1183 and absorbancy value of quercetin solution 0.1007. So, Inhibition percentage of standard 14.88%.

Endophytic fungi : Isolates A.Ps.1F, A.Ps.4F, A.Ps.5F and B.Ps.1F from stem of Piper betel plant had inhibition value of more than 50% of both the filtrate and biomass so the potential to be developed as isolates can produce active antidiabetic compounds. Most isolates from PDA media had a value of inhibition of less than 50%, so that no potential as a producer of antidiabetic compounds.

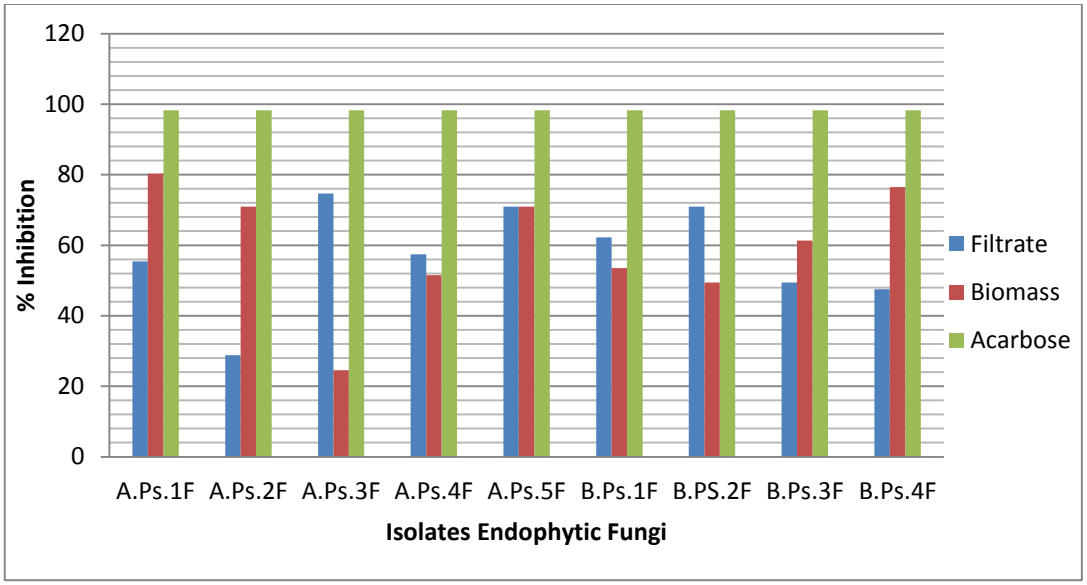

Figure 2. Inhibition α Glucosidase Isolates Endophytic Fungi and Acarbose

The antioxidant activity of endophytic fungi isolates from stem of Piper betel plant

The results of antioxidant activity test of endophytic fungi isolates of the Piper betel plant stem with Free radical scavenging method with DPPH reagents are presented in Table 3.

Most isolates fungus from Piper betel L plant does not have antioxidant activity. IC_{50} value isolates fungus is greater than 100 ppm so inactive as an antioxidant. Fungus A.Ps.5F have IC_{50} of 95.09 ppm to be the only one fungus that has potential as an antioxidant.

Nabasree Dasgupta and Bratati De also conducts research antioxidant activity using the free radicals scavenging by DPPH solvent to extract water from the leaves of the Piper betel plant three types in India, which is kind of kauri, ghanagete and bagerhati. Their results were not all of the water extract of this plant has antioxidant activity, just the kind of kauri potential as an antioxidant with IC_{50} values are 62.6 ppm, but the type ghanagete and bagerhati no potential with IC_{50} value of 126.0 ppm and 271.5 ppm (Nabsree D and Bratati De,2004).

Table 3. The results of antioxidant activity of endophytic fungi isolates from stem of Piper betel

No	Plant	Fungi Isolate	IC_{50}	Note
1	Piper betel L	A.Ps.1F	188,08	Not Active
2	Piper betel L	A.Ps.2F	126,10	Not Active
3	Piper betel L	A.Ps.3F	437,99	Not Active
4	Piper betel L	A.Ps.4F	125,89	Not Active
5	Piper betel L	A.Ps.5F	95,09	Active
6	Piper betel L	B.Ps.1F	161,03	Not Active
7	Piper betel L	B.Ps.2F	140,46	Not Active
8	Piper betel L	B.Ps.3F	363,62	Not Active
9	Piper betel L	B.Ps.4F	130,72	Not Active
10	Ascorbic acid		3.24	Control positive

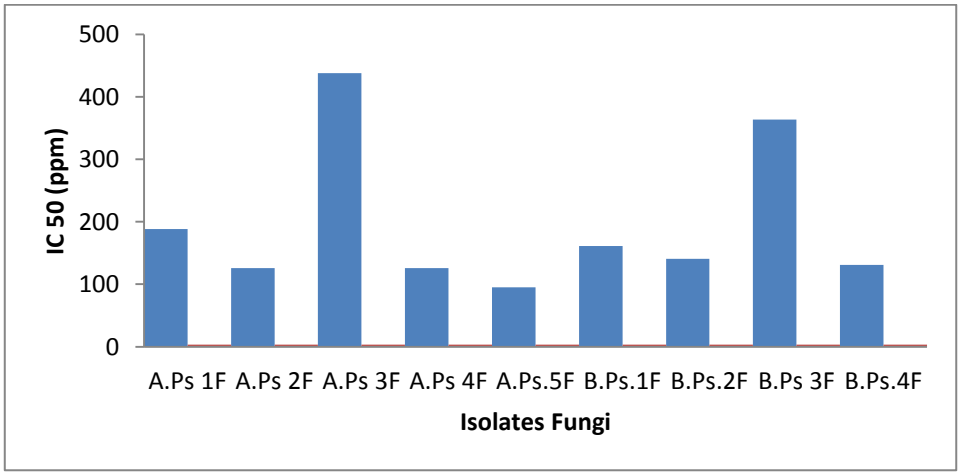

Figure 3. Antioxidant Activity Isolate Endophytic and Ascorbic Acid as Control positive

The antioxidant activity tested of isolates endophytic fungi from stem of Piper betel L by the free radical scavenging method using DPPH reagents and Ascorbic acid as a control positive. IC_{50} Ascorbic acid is 3.24 ppm. Result antioxidant test isolates this plant and Ascorbic acid are presented in Table 3. and Figure 3. IC_{50} reflects the size concentrations of test compound to capture radicals by 50%. IC_{50} obtained using the equation stating linear regression relationship the concentration of the sample (symbol x) with the activity of radical catcher (Symbol y) of a series of replicate measurements. The smaller the IC_{50} value of the test compound.The effectiveness have voted catcher radically better (Cholisoh and Utami, 2008).

Antidiabetic and antioxidant activity of endophytic fungi isolates of stem of Piper betel L plant

In this study, isolates of endophytic fungi are fermented in the PDA and CMM medium and obtained some endophytic fungi have a good antidiabetic activity of extracts filtrate and biomass, as shown in Figure 2. Isolate the endophytic fungi, among others A.Ps.1F (72, 59% and 92.22%), A.Ps.4F (81.87% and 79.37%), A.Ps.5F (63.40% and 96.09%) and B.Ps.1F (65 , 60% and 62.72%). The isolate A.Ps.5F is the only one of endophytic fungi from stem of Paper betel L active as an antioxidant with IC_{50} of 95.04 (<100). The isolates fungi also had a high antidiabetic activity that is potentially as antidiabetic agents and antioxidants that can produce antidiabetic and antioxidants compounds. While the results of other endophytic fungi isolates IC_{50} greater than 100 so inactive as an antidiabetic, as shown in Figure 3.

CONCLUSION

Isolation of endophytic fungi from stem of Piper betel plant on CMM and PDA media, obtained 9 isolates. Endophytic fungi A.Ps.5F active as an antioxidant with IC_{50} of 95.04 ppm and also have antidiabetic activity with the filtrate and biomass extract respectively for 70.92% and 70.92%, potentially as antidiabetic agents and antioxidants.

ACKNOWLEDGEMENTS

We express our appreciation to Prof.Dr.Partomuan Simanjuntak for their support in the implementation of research at Biotechnology Research Center, LIPI.

REFERENCES

A. A. L. Gunatilaka, “Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence,” Journal of Natural Products, vol. 69, no. 3, pp. 509–526, 2006. View at Publisher · View at Google Scholar View at Scopus.

Anuree Khanbun. 2004. Bioactive compounds from endophytic fungi isolated from Piper betle Linn.Chunglakorn University Intellectual Repository.Copyright © 2002-2005

Croizer, J., S.E. Thomas., M.C. Awie., H.C. Evan & A.A. Holmes. (2006). Molecular characterization of fungal endophytic morphospecies isolated from stem and pods of Theobroma cacao. Plant Pathology. 55, 783-791.

D. J. Newman and G. M. Cragg, “Natural products as sources of new drugs over the last 25 years,” Journal of Natural Products, vol. 70, no. 3, pp. 461–477, 2007. View at Publisher · View at Google Scholar · View at Scopus

Erawaty. (2012).Uji Aktivitas Antioksidan Ekstrak daun Garcinia daedalamthera Pierre dengan metode DPPH (1,1 difenil pikrihidrazil) dan Identifikasi golongan senyawa dari fraksi palingaktif. Skripsi F.MIPA Universitas Indonesia

G. A. Strobel, “Endophytes as sources of bioactive products,” Microbes and Infection, vol. 5, no. 6, pp. 535–544, 2003. View at Publisher · View at Google Scholar · View at Scopus

Halliwel, B. (2007). Dietary polyphenols: good, bad, or indifferent for your health. Cardiovascular Research J.73,341-347.

Kogel,K.H, Franken, P, H¨uckelhoven, R. (2006).Endophyte or parasite—what decides?” Current Opinion in Plant Biology,vol. 9, no. 4, pp. 358–363.

Kushagra Nagory, Mukesh, K.Singh, Amit Alexander.Piper betle L.: A review on its ethnobotany, phytochemistry, pharmacological profile and profiling by new hyphenated

technique DART-MS (Direct Analysis in Real Time Mass Spectrometry). Article · Jul 2016 · South African Journal of Botany

Li, J, Zhao, G.Z, Chen, H.H. (2008). Antitumour and antimicrobial activities of endophytic streptomycetes from pharmaceutical plants in rainforest. Letters in Applied Microbiology, vol. 47, no. 6, pp. 574–580.

Nabasree Dasgupta dan Bratati De. 2004. Antioxidant activity of Piper betle L. leaf extract in vitro.Elsevier. Food Chemistry. 88, (2), pp.219–224. Copyright © 2004 Elsevier Ltd.

N. L. Owen and N. Hundley, "Endophytes—the chemical synthesizers inside plants," Science Progress, vol. 87, no. 2, pp. 79–99, 2004. View at Scopus.

Oberley, L.W. (1998). Free Radicals and Diabetes. Free Radic. Biol Med. 5(2), 113-124

Onifade, A.K. (2007). Research trends: Bioactive metabolites of fungal origin. Res. J. of Biol. Scie. 2 (1), 81-84.

R. X. Tan and W. X. Zou, "Endophytes: a rich source of functional metabolites," Natural Product Reports, vol. 18, no. 4, pp. 448–459, 2001. View at Publisher · View at Google Scholar · View at Scopus

Saijyo, J., Suzuki, Y., Okuno, Y., & Yamaki, H. (2008). α-glucosidase Inhibitor from Bergenia Ligulata. J.Oleo Sci. 57 (8), 431-435.

Strobel, G. & Daisy, B. (2003) Bioprospecting for microbial endophytes and their natural products. Microbiology and molecular biology reviews 67, 491–502.

Strobel, G.A. (2004) "Endophytes as sources of bioactive products," Microbes and Infection, vol. 5, no. 6, pp. 535–544.

Suarsana ,I. N., Priosoeryanto., Bintang M,. Wrediyanti T., 2008., Aktivitas Daya Hambat Enzim a-glukosidase dan Efek Hipoglikemik Ekstrak Tempe pada Tikus Diabetes., J.Veteriner : 9 (3) : 122-127

Sunil C., Gopalakrishnan L., Palanisamy M., Kuliya M.K., Paul A. 2009., α-glucosidase inhibitory and antidiabetic activities of ethanolic extract of Pisonia alba Spam leaves. Int. J. of Integrative Biol. 6 (1) : 41-45

Tiwary, A.K., & Rao, J.M. (2002). Diabetes Melitus and Multiple Therapeutic Approaches of Phytochemicals: Present Status and Future Prospect.Current Science. 83(1), 30-38.

Theantana, T., Hyde, K.O., & Lumyong, A.(2007). Asparaginase production by endophytic fungi isolated from some thai medical plants. KMITL, Scie.Tech. J. 7 (1), 13-18.

WHO Media Centre (2016)

Zhang, L, An, R, Wang, J. (2005)."Exploring novel bioactive compounds from marine microbes," Current Opinion in Microbiology, vol. 8, no. 3, pp. 276–281.


Design By : Hanter Olong