BIOSFER: Jurnal Tadris Biologi Vol. 12 No. 1 (2021) 65-68

Contents lists available at BIOSFER

BIOSFER: JURNAL TADRIS BIOLOGI

p-ISSN: 2086-5945 (print), e-ISSN: 2580-4960 (online), DOI 10.24042/biosfer

http://ejournal.radenintan.ac.id/index.php/biosfer/index

ANTAGONISTIC ACTIVITY OF THE ENDOPHY FUNGI OF LIME PLANT (CITRUS AURANTIFOLIA) ON CANDIDA ALBICANS

Oktira Roka Aji[1]*, Sri Wahyuni[2]

[1, 2] Universitas Ahmad Dahlan, Yogyakarta, Indonesia

ARTICLE INFO

Article History

Received: 15-04-2021
Accepted: 18-05-2021
Published: 30-06-2021

Keywords:

Citrus aurantifolia;
Endophytic fungi;
Candida albicans.

Correspondence email:
oktira.aji@bio.uad.ac.id

ABSTRACT

This study aims to isolate and determine the antagonistic activity of lime's endophytic mushrooms on Candida albicans. Antagonism tests were performed in vitro with dual culture methods. The study obtained 14 isolates that were successfully isolated from the stem, roots, leaves, and peels of lime fruit. A total of 8 isolates could inhibit Candida albicans through antibiosis mechanisms and healthy competition. The isolates with the highest inhibition percentage were KD2 and MA1 (42.85%).

Aktivitas Antagonistik Jamur Endofit Tanaman Jeruk Nipis (*Citrus aurantifolia*) Terhadap *Candida albicans*

ABSTRAK: *Penelitian ini bertujuan untuk mengisolasi dan mengetahui aktivitas antagonistik jamur endofit dari jeruk nipis terhadap Candida albicans. Uji antagonisme dilakukan secara in vitro dengan metode uji ganda (dual culture). Berdasarkan hasil penelitian diperoleh 14 isolat yang berhasil diisolasi dari organ batang, akar, daun dan kulit buah jeruk nipis. Sebanyak 8 isolat diantaranya memiliki kemampuan menghambat Candida albicans melalui mekanisme antibiosis dan kompetisi nutrisi. Isolat yang memiliki persentase hambatan paling tinggi adalah isolat KD2 dan MA1 yaitu sebesar 42,85%.*

INTRODUCTION

Indonesia is an agricultural country (Hijjang et al., 2014) famous for its agricultural products, including lime (IKASARI et al., 2018; Putra et al., 2019). Lime (*Citrus aurantifolia*) is one of the plants that is often used as herbal medicine (Chismirina & Magistra, 2016; Parama et al., 2019; Razak et al., 2013). However, endophytic microbes in lime plants are not widely known. Endophytic microbes can be fungi or bacteria found in healthy plant tissue without causing disease symptoms (Kurnia et al., 2014; Puspita et al., 2013). The endophytic fungi occupy roots, stems, bark, leaves, and fruits (Utami, 2017).

Endophytic fungi have not been widely explored (Puspita et al., 2013). It is estimated that there are at least one million species of endophytic fungi (Suliati, n.d.; Yulianti, 2012). Endophytic fungi are known to inhibit the growth of several pathogenic fungi, namely *Cryptococcus neoformans* and *Candida albicans* (Kuncoro & Sugijanto, 2011). Endophytic fungi inhibit the growth of other microbes through the mechanism of nutrient competition, antibiosis and parasitism

(Amaria et al., 2016; Kusumawardani et al., 2015; Rachmatunnisa & Rukmi, 2017). Endophytic fungi produce bioactive compounds that can inhibit the growth of pathogenic microbes (Iqlima et al., 2017; Sepriana et al., 2020; Widiantini et al., 2018). These compounds also can be developed as new antimicrobial candidates (Astuti et al., 2019; Suardana et al., 2007).

Furthermore, Candida albicans is a normal flora found in the gastrointestinal tract (Itsa et al., 2018; Makhfirah et al., 2020), skin, and mucous membranes (Kurniawati et al., 2020; Pertiwi et al., 2016). However, an excessive amount of Candida albicans can cause Candidiasis (Alfiah et al., 2015; Lestari, 2015; Mutia & Saleh, 2016). Candidiasis is a disease that can attack humans' skin, nails, and mucous membranes (Indrayati & Sari, 2018). Candida Albicans high resistance level to antifungals, such as amphotericin-B and fluconazole, makes the development of new antimicrobials urgent (Imani, 2014; Septiadi et al., 2013).

Many studies on antagonistic activity have been conducted, including the antagonistic activity of ginger endophytic fungus and compost solution (Sari et al., n.d.; Suardana et al., 2007), administration of growth regulators on limes' shoot growth (Aeni et al., 2017), and the In Vitro growth of Staphylococcus aureus bacteria (Razak et al., 2013). The previous research focused on the fungal analysis, not the antagonistic activity. Therefore, in this study, the lime's endophytic fungi were isolated and tested for isolates' antagonistic activity on the in-vitro growth of the pathogenic fungus Candida albicans.

METHOD

The lime plants were obtained from the garden in Warungboto, Yogyakarta, taken in April. The plant organs taken were the roots, stems, leaves, and peels. The researchers performed the fungal isolation process in a laboratory. The roots, stems, leaves, and peels of lime fruit were cut into 2 cm parts. Each sample was washed with 70% alcohol for 30 minutes and then washed with NaCIO solution for 60 seconds. Next, the samples were washed again with alcohol for 30 seconds, washed with sterile distilled water, and dried. Each sample was then ground and placed on PDA media to grow the fungal colonies. After the fungus grows, it is then transferred to a new medium to be observed macroscopically and microscopically.

The tests performed were the dual test method (dual culture). Pure Candida albicans and each isolate of endophytic fungus were inoculated on a petri dish containing PDA media with a distance of 3 cm between the endophytic fungus and Candida albicans. The negative control was Candida albicans without endophytic fungi inoculation. They were incubated at room temperature (25- 28°C) for seven days. Observations were made by measuring the diameter of fungal growth and the inhibition zone formed.

RESULTS AND DISCUSSION

Fourteen fungal isolates were obtained as the results of the endophytic fungi isolation. The macroscopic characteristics of fungi can be seen in Table 1, while the microscopic characteristics of fungi can be seen in Table 2. Each fungus had different characteristics. Macroscopic observations were made to observe the colour of the fungal colonies, while microscopic observations were made to observe the shape of the hyphae and the means of fungal reproduction. Endophytic fungi with codes MA1, MB1, MC1, KA2, and KB1 had septate hyphae, while isolates MA2, MB2, MC2, KA1, KB1, KC1, KC2, KD1 and KD2 had non-septate hyphae. The morphological differences of the endophytic fungal hyphae can be seen in Figure 1. The asexual reproduction of endophytic fungi isolates in lime has different forms.

Table 1. Macroscopic Characteristics of Endophytic Fungal Isolates.

No.	Origin Organ	Code	Top Colony Color	Bottom Colony Color
1.	Root	MA1	White	Yellow
		MA2	Green	White
		KA1	Black	Yellow
		KA2	Gray	White
2.	stem	MB1	White	Beige
		MB2	Green	White
		KB1	White	Chocolate
		KB2	Green	White
3.	Peel	MC1	Green	White
		MC2	White	White
		KC1	Yellow	White
		KC2	Black	White
4.	Leaf	KD1	Green	Yellow
		KD2	Black	Yellow

Table 2. Microscopic Characteristics of Endophytic Fungal Isolates

No.	Origin Organ	Code	Hyphae Form	Spore form/conidia
1.	Root	MA1	Septate	Crescent moon
		MA2	Non-septate	Round
		KA1	Septate	Oval
		KA2	Non-septate	Round
2.	stem	MB1	Non-septate	Round
		MB2	Non-septate	Round
		KB1	Septate	Round conidia
		KB2	Septate	Round conidia
3.	Peel	MC1	Septate	Oval
		MC2	Non-septate	Round
		KC1	Non-septate	Round
		KC2	Non-septate	Round
4.	Leaf	KD1	Non-septate	Round
		KD2	Non-septate	Round

The antagonism test revealed that eight isolates showed antagonistic activity. The inhibition differences of each endophytic fungal isolate on Candida albicans can be seen in Figure 2. The inhibition percentage was calculated by comparing the length of the radius of Candida albicans colonies between the control and the treatment using the dual culture method. The highest endophytic fungi inhibition was found in isolates KD2 and MA1 (42.85%). The fungus that had the lowest percentage of inhibition was KC1 (26.67%).

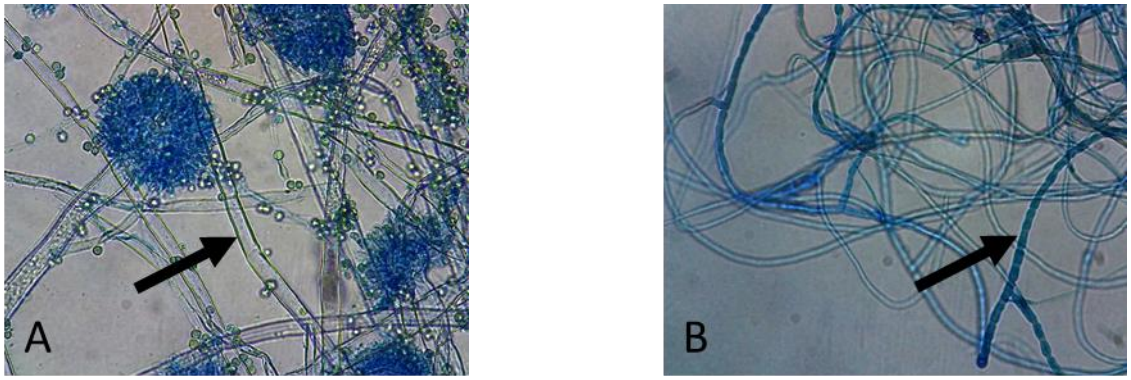

Figure 1. Microscopic Observation of Endophytic Fungal Isolates (A) Septate Hyphae on KD1 Isolates (B) Septate Hyphae on KB1 isolates (400x Magnification)

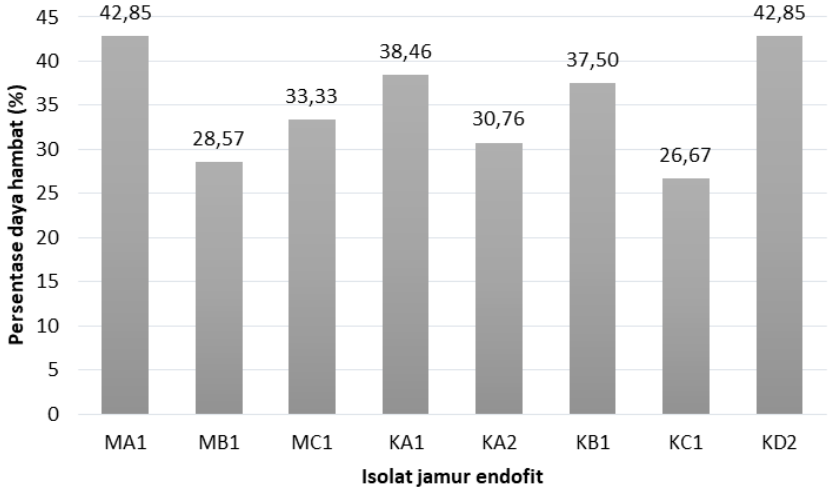

Figure 2. The Inhibition Percentage of Endophytic Fungi on Candida Albicans

The inhibition mechanism can be antibiosis and nutritional competition. Observations on the antagonism test showed that isolates MA1, MC1, KAI, and KD2 showed an antibiosis mechanism of antagonism because an inhibitory zone was formed between mycelium. The mechanism of inhibition can be seen in Figure 3. The mechanism of antibiosis was indicated by the clear zones formed between colonies of endophytic fungi and Candida albicans. The antibiosis mechanism can be determined by forming a clear zone of inhibition around the colonies of antagonistic fungi and pathogenic fungi (Imu Rohayatun & Rahmawati, 2017). In the antibiosis inhibition mechanism, fungi secrete bioactive compounds to suppress the growth of other nearby fungi (Ridwan & Prastia, 2017). Fungi can produce alkaloids and mycotoxins that can damage the growth of other fungi (Dani et al., 2012; Kusumawardani et al., 2015). Therefore, the inhibition zone was caused by the production of bioactive compounds that inhibited the growth of other fungi.

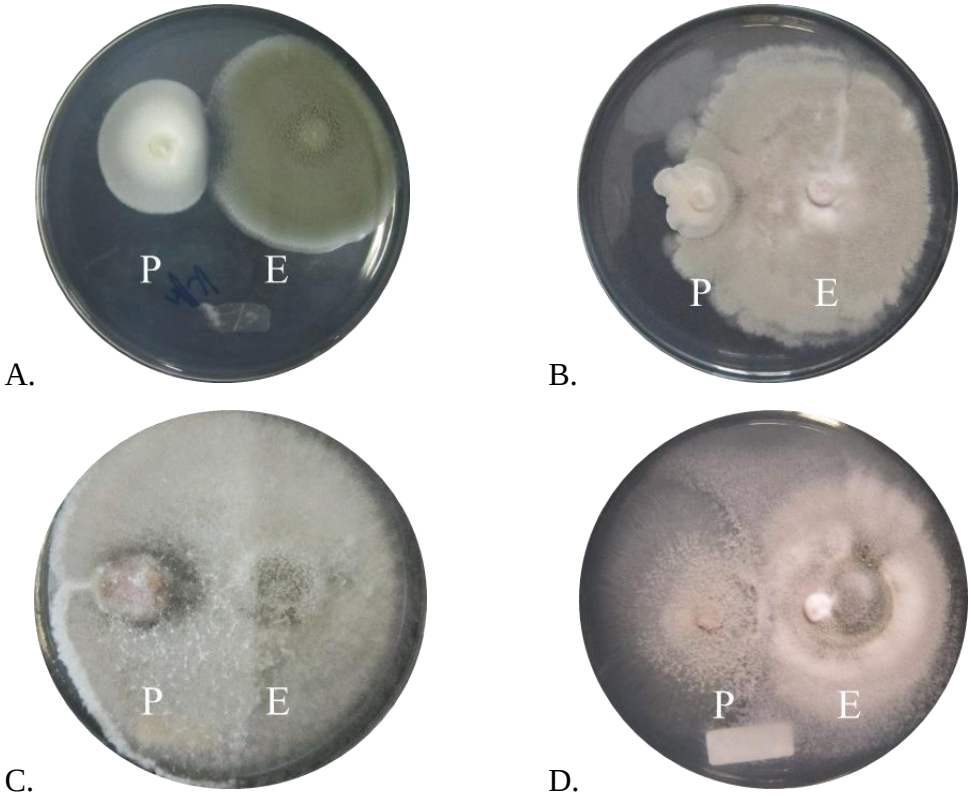

Figure 3. The results of the antagonism test between endophytic fungi and Candida albicans. (A.) An inhibition zone was formed between endophytic fungi (KA1) and Candida albicans, (B.) Endophytic fungi (KC1) inhibited Candida albicans without forming an inhibition zone. (C.) Endophytic fungi (KB1) covered the colonies of Candida albicans, (D.) Endophytic fungi (MB1) grew faster than Candida albicans. Description : P = Candida albicans fungus; E = Endophytic fungi.

Another antagonistic mechanism is competition for nutrients. Isolates MB1, KA2, KB1, and KC1, showed an antagonism mechanism by nutritional competition. The mechanism of nutrient competition was caused by differences in growth rates between endophytic fungi and Candida albicans on PDA media. The mechanism of nutrient competition occurred because of the nutritional requirements of fungi and different growth rates. Also, the third indicator described the relationship and created a model. In this analysis, the error made was that the lime plant studied was not only the Candida albicans fungus, according to the problem statement.

Moreover, the fungus is also present in other plants. Based on the results of interviews, the errors made by research subjects were generally caused by several factors (Kurniawati et al., 2020) and the role of virulence factors in Candida Albicans (Lestari, 2015). The results also revealed that there were many differences and shortcomings from other studies. Besides the material misunderstanding and testing tools, it was impossible to determine the other fungal shapes based on the size and shape of a particular lime.

CONCLUSIONS AND SUGGESTIONS

Fourteen endophytic fungi isolated from lime (Citrus aurantifolia) were obtained from root organs, stems, leaves, and peels. The fungal isolates that produced the highest percentage of inhibition were KD2 and MA1 (42.85%). The inhibition mechanism of the endophytic fungi on Candida albicans was antibiosis and nutritional competition. Further research is needed to identify endophytic fungi with a molecular approach. The researchers suggest further research must provide appropriate trial material.

REFERENCES

Aeni, N., Salman, S., & Sukmasari, M. D. (2017). Cara perbanyakan vegetatif dan pemberian zat pengatur tumbuh terhadap pertumbuhan tunas pada tanaman jeruk nipis (Citrus aurantifolia swingle). *Agrivet Journal*, *5*(2).

Alfiah, R. R., Khotimah, S., & Turnip, M. (2015). Efektivitas ekstrak metanol daun sembung rambat (Mikania micrantha Kunth) terhadap pertumbuhan jamur Candida albicans. *Protobiont*, *4*(1).

Amaria, W., Harni, R., & Samsudin, S. (2016). Evaluation of Antagonistic Fungi in Inhibiting The Growth of Rigidoporus microporus Causing White Root Disease in Rubber Plants. *Jurnal Tanaman Industri Dan Penyegar*, *2*(1), 51–60.

Astuti, P., Alam, G., Pratiwi, S., Hertiani, T., & Wahyuono, S. (2019). Skrining Senyawa Antiinfeksi dari Spons yang Dikoleksi dari Bunaken, Manado. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*, *8*(2), 47–52.

Chismirina, S., & Magistra, R. Y. (2016). Konsentrasi hambat dan bunuh minimum ekstrak daun jeruk nipis (citrus aurantifolia) terhadap aggregatibacter actinomycetemcomitans secara in vitro. *Cakradonya Dental Journal*, *8*(1), 68–76.

Dani, I. W., Nurtjahja, K., & Zuhra, C. F. (2012). Penghambatan pertumbuhan Aspergillus flavus dan Fusarium moniliforme oleh ekstrak salam (Eugenia polyantha) dan kunyit (Curcuma domestica). *Saintia Biologi*, *1*(1), 8–14.

Hijjang, P., Lampe, M., & Basir, M. (2014). Aneka ragam pengetahuan lokal dan kreatifitas petani yang mendukung agroecopreneuer ramah lingkungan di Sulawesi Selatan. *Sosiohumaniora*, *16*(2), 143–148.

Ikasari, D. M., Nurika, I., Meidiana, C., & Damayanti, R. (2018). Upaya pengembangan desa agrowisata melalui penguatan agroindustri terpadu produk unggulan daerah (buah naga dan jeruk siam) di Desa Temurejo, Banyuwangi. *Prosiding Semnas PPM 2018*, *1*(1), 1397–1405.

Imani, A. Z. (2014). Uji Aktivitas Antijamur Ekstrak Etanol Daun Mangga Bacang (Mangifera foetida L.) Terhadap Candida albicans Secara In Vitro. *Jurnal Mahasiswa PSPD FK Universitas Tanjungpura*, *3*(1).

Imu Rohayatun, M., & Rahmawati, M. (2017). Uji Antagonis Isolat Jamur Rizosfer Lokal Terhadap Phytophthora sp. Im5 dari Pangkal Batang Tanaman Jeruk Siam (Citrus nobilis var. Microcarpa). *Protobiont*, *6*(2).

Indrayati, S., & Sari, R. I. (2018). Gambaran Candida albicans Pada bak Penampung Air di Toilet SDN 17 Batu Banyak Kabupaten Solok. *Jurnal Kesehatan Perintis*, *5*(2), 159–164.

Iqlima, D., Ardiningsih, P., & Wibowo, M. A. (2017). Aktivitas antibakteri isolat bakteri endofit B2D dari batang tanaman yakon (Smallanthus sonchifolius (Poepp. & Endl.) H. Rob.) terhadap bakteri Staphylococcus aureus dan Salmonella thypimurium. *Jurnal Kimia Khatulistiwa*, *7*(1).

Itsa, N. S., Sukohar, A., & Anggraini, D. I. (2018). Pemanfaatan Cuka Sari Apel Sebagai Terapi Antifungi Terhadap Infeksi Candida albicans (Kandidiasis). *Jurnal Majority*, *7*(3), 290–295.

Kuncoro, H., & Sugijanto, N. E. (2011). Jamur Endofit, Biodiversitas, Potensi Dan Prospek Penggunaannya Sebagai Sumber Bahan Obat Baru. *Journal of*

Tropical Pharmacy and Chemistry, *1*(3), 247–262.

Kurnia, A. T., Pinem, M. I., & Oemry, S. (2014). Penggunaan Jamur Endofit untuk Mengendalikan Fusarium oxysporum f. Sp. Capsici dan Alternaria solani Secara in Vitro. *Jurnal Agroekoteknologi Universitas Sumatera Utara*, *2*(4), 102117.

Kurniawati, D., Noval, N., & Nastiti, K. (2020). Potensi antiseptik poliherbal daun sirih (piper betle), kulit jeruk nipis (citrus aurantifolia) dan tanaman bundung (actinuscirpus grossus) pada tindakan keperawatan dan kebidanan. *Dinamika Kesehatan: Jurnal Kebidanan Dan Keperawatan*, *11*(1), 420–431.

Kusumawardani, Y., Sulistyowati, L., & Cholil, A. (2015). Potensi antagonis jamur endofit pada tanaman lada (Piper nigrum L.) terhadap jamur Phytophthora capsici Leionian penyebab penyakit busuk pangkal batang. *Jurnal Hama Dan Penyakit Tumbuhan*, *3*(1), 21–29.

Lestari, P. E. (2015). Peran faktor virulensi pada patogenesis infeksi Candida albicans. *STOMATOGNATIC-Jurnal Kedokteran Gigi*, *7*(2), 113–117.

Makhfirah, N., Fatimatuzzahra, C., Mardina, V., & Hakim, R. F. (2020). Pemanfaatan Bahan Alami Sebagai Upaya Penghambat Candida Albicans Pada Rongga Mulut. *Jurnal Jeumpa*, *7*(2), 400–413.

Mutia, U., & Saleh, C. (2016). Uji Kadar Asam Laktat Pada Keju Kacang Tanah (Arachis Hypogaea L.) Berdasarkan Variasi Waktu Dan Konsentrasi Bakteri Lactobacillus Bulgaricus Dan Streptococcus Lactis. *Jurnal Kimia Mulawarman*, *10*(2).

Parama, P. W., Sukrama, I. D. M., & Handoko, S. A. (2019). Uji efektifitas antibakteri ekstrak buah jeruk nipis (Citrus aurantifolia) terhadap pertumbuhan Streptococcus mutans in vitro. *Bali Dental Journal*, *3*(1), 45–52.

Pertiwi, R. D., Kristanto, J., & Praptiwi, G. A. (2016). Uji aktivitas antibakteri formulasi gel untuk sariawan dari ekstrak daun saga (Abrus precatorius Linn.) terhadap bakteri Staphylococcus aureus. *Jurnal Ilmiah Manuntung: Sains Farmasi Dan Kesehatan*, *2*(2), 239–247.

Puspita, Y. D., Sulistyowati, L., & Djauhari, S. (2013). Eksplorasi Jamur Endofit Pada Tanaman Jeruk (Citrus Sp.) Fusiprotoplas Dengan Ketahanan Berbeda Terhadap Botriodiplodia theobromae Pat. *Jurnal Hama Dan Penyakit Tumbuhan*, *1*(3).

Putra, G. A., Raessi, S., & Mahdi, M. (2019). Analisis Kelayakan Finansial Usaha Perkebunan Jeruk Nipis (Citrus Aurantifolia Swingle) di Nagari Padang Gantiang Kecamatan Padang Ganting Kabupaten Tanah Datar. *JOSETA: Journal of Socio-Economics on Tropical Agriculture*, *1*(1).

Rachmatunnisa, R., & Rukmi, M. I. (2017). Aktivitas Antagonistik Kapang Endofit Duwet (Syzygium cumini (L.) Skeels) Terhadap Alternaria porri Penyebab Bercak Ungu pada Bawang Merah (Allium ascalonicum L.) secara In-Vitro. *Jurnal Akademika Biologi*, *6*(1), 71–78.

Razak, A., Djamal, A., & Revilla, G. (2013). Uji daya hambat air perasan buah jeruk nipis (Citrus aurantifolia s.) terhadap pertumbuhan bakteri Staphylococcus Aureus secara In Vitro. *Jurnal Kesehatan Andalas*, *2*(1), 05–08.

Ridwan, M., & Prastia, B. (2017). Pemamfaatan Tiga Jenis Pestisida Nabati untuk Mengendalikan Hama Kutu Daun Penyebab penyakit Kriting Daun pada Tanaman Cabe Merah. *Jurnal Sains Agro*, *2*(1).

Sari, N. K. Y., Kawuri, R., & Parwanayoni, N. M. S. (n.d.). *Aktivitas Antibakteri Fungi Endofit dari Rimpang Jahe Gajah (Zingiber officinale var. Roscoe) terhadap Methicillin Resistant Staphylococcus aureus (MRSA) Antibacterial Activity of Endophytic Fungi from Elephant Ginger Rhizome (Zingiber officinale var. Roscoe) Against Methicillin Resistant Staphylococcus aureus (MRSA)*.

Sepriana, C., Sumiati, E., Jekti, D. S. D., & Zulkifli, L. (2020). Identifikasi dan Uji Daya Hambat Isolat Bakteri Endofit Bunga Tanaman Cengkeh (Syzygium aromaticum L.) Terhadap Bakteri Patogen. *Jurnal Penelitian Pendidikan IPA*, *6*(1), 101–106.

Septiadi, T., Pringgenies, D., & Radjasa, O. K. (2013). Uji fitokimia dan aktivitas antijamur ekstrak teripang keling (Holoturia atra) dari pantai Bandengan Jepara terhadap jamur Candida albicans. *Journal of Marine Research*, *2*(2), 76–84.

Suardana, I. W., Suarsana, I. N., Sujaya, I. N., & Wiryawan, K. G. (2007). Isolasi dan identifikasi bakteri asam laktat dari cairan rumen sapi bali sebagai kandidat biopreservatif. *Jurnal Veteriner*, *8*(4), 155–159.

Suliati, R. (n.d.). Jenis-Jenis Jamur Endofit Tanaman Jeruk Siam (Citrus nobilis var. Microcarpa) di Perkebunan Dungun Prapakan Sambas. *Protobiont*, *6*(3).

Utami, D. P. (2017). Isolasi, Identifikasi Dan Aktivitas Bakteri Endofit Daun Kemangi (Ocimum Basilicum L.) Terhadap Staphylococcus aureus. *Jurnal Mahasiswa PSPD FK Universitas Tanjungpura*, *4*(1).

Widiantini, F., Yulia, E., & Nasahi, C. (2018). Potensi antagonisme senyawa metabolit sekunder asal bakteri endofit dengan pelarut metanol terhadap jamur G. boninense Pat. *Agrikultura*, *29*(1), 55–60.

Yulianti, T. (2012). Menggali potensi endofit untuk meningkatkan kesehatan tanaman tebu mendukung peningkatan produksi gula. *Jurnal Perspektif*, *11*(2), 111–122.