Proceedings of International Joint Seminar
UPSI – LSM SHARING, 11 Dec 2023
Vol.1 No. 1 (2024): 109-119

ISOLATION AND CHARACTERIZATION OF ENDOPHYTE MICROBES, AND PHYTOCHEMICAL TESTING OF THE RHIZOMES OF THE SQUIRREL'S HEAD FERN (DRYNARIA QUERCIFOLIA J. SM)

Rosdarni[1*], Sri Anggarini Rasyid[1], Desak Ketut Sutiari[1], Ridwan Adi Surya[2], Wa Ode Monalisa[1]

[1]Universitas Mandala Waluya
Jl. Jendral AH. Nasution, Kambu, Kec. Kambu, Kota Kendari, Sulawesi Tenggara. 93561 - Indonesia
[2]Universitas Halu Oleo, Sulawesi Tenggara – Indonesia

*E-mail: rosdarni@gmail.com

ABSTRACT

The rhizomes of the Squirrel's Head Fern are utilised as traditional medicine in various regions due to their perceived medicinal properties, akin to other herbs. Coevolution with endophytic microbes has rendered this compound with antimicrobial, anti-inflammatory, and anti-diabetic attributes. This study aims to isolate and characterise endophytic microbes, as well as screen the phytochemicals present in the rhizomes of the Squirrel's Head Fern. The research methodology encompassed three stages: isolation, macroscopic and microscopic characterization of the isolates, and analysis of their biochemical properties. The final stage involved phytochemical screening. Macroscopically, parameters such as colony shape, surface size, edges, and colour were examined, with a focus on bacteria. For fungal isolates, the carbohydrate fermentation test was conducted. The findings revealed five isolates, comprising three bacterial (MB1 from the genus Streptobacillus, MB2 from the genus Bacillus, and MB4 from the genus Corynebacterium) and two fungal (MJ1 and MJ2 from the genus Aspergillus) isolates. Phytochemical screening of the extract from the rhizomes of the Squirrel's Head Fern indicated the presence of flavonoids, phenols, alkaloids, saponins, tannins, and triterpenoids.In conclusion, MB1, MB2, and MB4 belong to the genera Streptobacillus, Bacillus, and Corynebacterium, respectively, while MJ1 and MJ2 belong to the genus Aspergillus. Further research is recommended to assess the antimicrobial activity of the isolates.

Keywords: *Isolation and Identification, Phytochemical Tests, Squirrel's Head Fern*

INTRODUCTION

The Squirrel's Head Fern (Drynaria quercifolia J. Sm) is an epiphytic plant that thrives on rocks, soil, and tree trunks. This plant is known for its medicinal compounds. Gupta et al. (2021) reported that its leaves can be utilised to treat tuberculosis, high fever, dyspepsia, increase appetite, and address chronic jaundice. Chatterjee et al. (2022) reported that administering Drynaria quercifolia J. Sm extract in alternation with paracetamol significantly reduced serum hepatotoxicity markers (ALT, AST, and ALP), renal toxicity (urea and creatinine), levels of lipid peroxidation, and histological damage to the liver and kidneys. Prasanna et al. (2019) reported its potential use as an antidiabetic. According to Nithin et al. (2020), the phytochemical content of

Drynaria quercifolia J. Sm includes glycosides, tannins, flavonoids, steroids, triterpenoids, carbohydrates, amino acids, and proteins.

Compounds found in plants can also be produced by endophytic microbes, organisms that reside within the plant, extracting nutrients without causing harm. These endophytic microbes can act as protectors against unfavourable conditions, exhibiting co-evolution with their host, thereby functioning as antimicrobials, anti-inflammatories, and antioxidants. Some endophytic microbes associated with plant tissues, such as Penicillium notatum and P. chrysogenum isolated from plants like ginger, turmeric, and red beans, can be used as antibiotics. Streptomyces bacteria, known as the largest producers of antibiotics, have been isolated by Febriansyah et al. (2021), who successfully identified manotriase from Streptomyces as an anti-breast cancer agent both in vivo and in vitro. Additionally, Suryani et al. (2020) reported that fumagisin, Aspergillate, and Clavicin compounds produced by A. fumigatus, A. flavus, and A. clavatus isolated from plant roots possess antimicrobial and anticancer properties.

The exploration of endophytic microbes producing specific compounds in the rhizomes of the Squirrel's Head Fern has not been conducted thus far. Empirical data indicate that in certain regions, such as Buton Utara Regency, the rhizomes of this plant is utilised as a herbal remedy for treating various ailments including fever, jaundice, vomiting blood, deep wound infections, bleeding, and lung injuries. This is done through boiling, direct consumption, or by grating the rhizome and then drinking the resulting water. Previous research has been limited to determining the presence or absence of bacterial and fungal endophytic microbes. For instance, Rusli and Rahmaniar (2013) successfully isolated five bacterial and five fungal isolates, but the identification of genera or even species was not undertaken. Therefore, this study aims to delve into the macroscopic, microscopic characteristics, and biochemical properties of the isolated microbes. Additionally, it would explore the phytochemical content present in the rhizomes of the Squirrel's Head Fern.

METHOD

This study adopted a qualitative approach with a laboratory exploration design. The research was conducted at the Microbiology Laboratory of D4 Medical Laboratory Technology and the Pharmacy Laboratory at Universitas Mandala Waluya. The methodology encompassed three stages: isolation, characterization, and phytochemical testing, each described as follows:

1. **Isolation Stage**

   The isolation process began with sample preparation, involving cleaning, dicing, and planting in NA and PDA media. Samples, sourced from Linsowu Village, Buton Utara Regency, a prominent user of plant rhizomes, were planted in duplicate on NA and PDA media, coded as MB1, MB2, MB3, and MB4 for NA media, and MJ1, MJ2, MJ3, and MJ4 for PDA media. Incubation at room temperature followed for 2x24 hours (NA media) and 5x24 hours (PDA media). Growing colonies were then observed, and purification of each colony was performed.

2. **Characterization Stage**

   Characterization of the isolates included macroscopic, microscopic, and biochemical assessments. Macroscopically, colonies were observed for shape, size, surface, edges, and colour. Microscopically, cell shape (bacillus, coccus, spiral, or vibrio) was observed, along with gram characteristics for bacteria and the shape of conidia, vesicles, conidiophores, and metulae for fungi through methylene blue staining. Biochemical properties were observed through indole, motility, H2S, TSIA, citrate, and catalase tests for bacteria, and carbohydrate fermentation and gelatin tests for fungi.

3. **Phytochemical Test Stage**

   Phytochemical tests were conducted to test the secondary metabolite content in the rhizomes of the Squirrel Head Fern. This series of tests included tests for flavonoids, phenolics, alkaloids, saponins, tannins, and triterpenoids. The flavonoid test involved the addition of concentrated MG + HCL, revealing positive results when an orange colour formed. The phenolic test involved the addition of FeCl3, revealing positive results when a bluish-green or dark blue colour formed. The alkaloid test involved the addition of HCL 2N + Dragendruf, revealing positive results when a yellowish-white precipitate formed. The saponin test involved the addition of H2O, revealing positive results when foam formed. The tannin test involved the addition of 1% FeCl3, revealing positive results when a bluish colour formed. Lastly, the Triterpenoid test involved the addition of anhydrous acetic acid and H2SO4, revealing positive results when a purple-brown colour formed. The data obtained were presented through tables and figures, followed by analysis and interpretation. Data analysis involved describing the results of the observations.

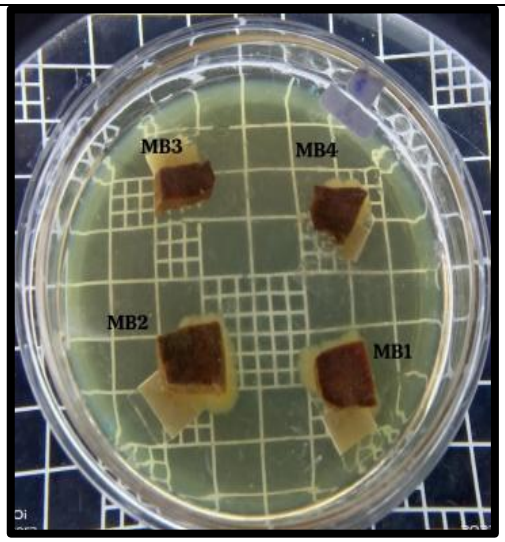
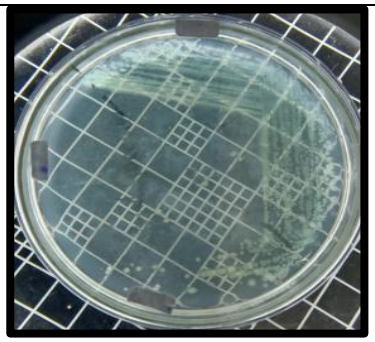
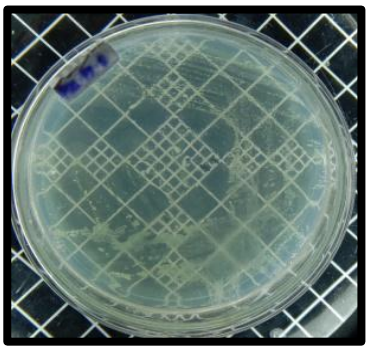
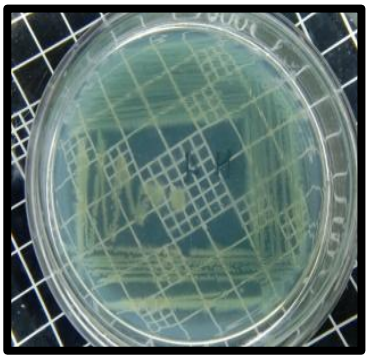
RESULT AND DISCUSSION

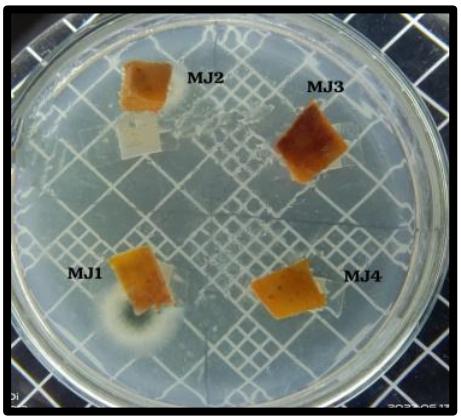
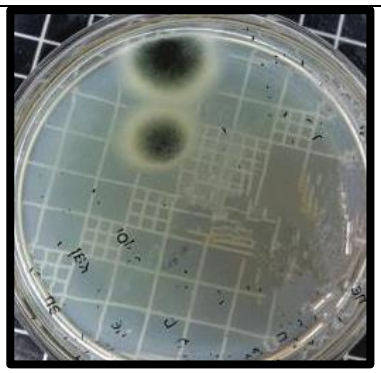
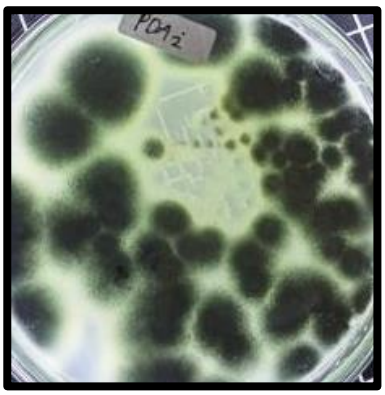
1. Result

a. Isolation of Endophytic Microbes

The successful isolation yielded five isolates, encompassing three isolates on NA media with codes MB1, MB2, and MB4, and two isolates on PDA media, with codes MJ1 and MJ2. Isolate codes MB3, MJ3, and MJ4 did not exhibit growth within the designated incubation period, indicating the presence of three bacterial isolates and two fungal isolates. The purification process resulted in five isolates, each exhibiting distinct characteristics as outlined in Table 1.

Table 1.
Results of Isolation of Endophytic Microbes in the Rhizomes of the Squirrel's Head Fern

No	Growth of Isolates on Both Media	Purification Results	Note
1		 **MB1 isolate**  **MB2 isolate**  **MB4 isolate**	Purification was conducted until the colonies were completely pure for each isolate, encompassing both bacterial and fungal colonies.

No	Growth of Isolates on Both Media	Purification Results	Note
2		 **MJ1 isolate**  **MJ2 isolate**	

b. Results of Microscopic, Macroscopic Characteristics and Biochemical Properties of the Isolates

The macroscopic characteristics results, obtained by observing colony characteristics, revealed that the MB1 isolate was of medium size, round (spherical) in shape, creamy white in colour, with flat edges and a convex surface. Regarding the MB2 isolate, the only difference lay in its size and colour—it was small and white. For MB4, it exhibited a small size, irregular shape, cream colour, flat edges, and a convex surface. Fungal isolates with codes MJ1 and MJ2 exhibited nearly identical colony characteristics, including a medium size, round shape, cotton-like surface, and a dark green reverse colony colour. The only difference was in the colony colour, with MJ1 exhibited a green colour with a white fringe, while MJ2 exhibited a dark green colour. These characteristics are detailed in Table 2.

Table 2.
Results of Macroscopic Characteristics of the Isolates

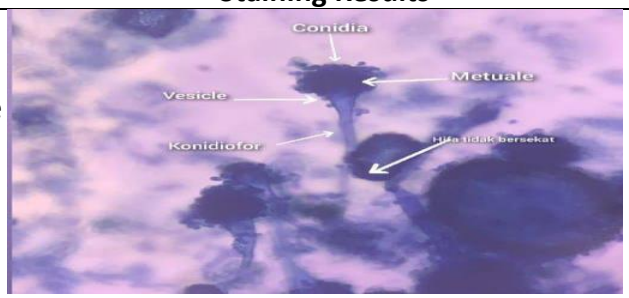
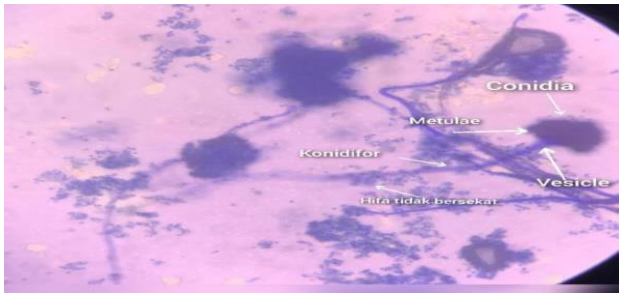
No	Bacterial Islotate Code	Macroscopic				
		Size	**Form**	**Colour**	**Periphery**	**Surface**
1	MB1	Medium	Round	Creamy white	Flat	Convex
2	MB2	Small	Round	White	Flat	Convex
3	MB4	Small	Irregular	Cream	Flat	Convex
No	**Fungal Isolate Code**	**Size**	**Form**	**Colony Colour**	**Surface Properties**	**Reverse Colour**
1	MJ1	Medium	Round	Green with a white fringe	Cotton-like	Dark green
2	MJ2	Medium	Round	Dark green	Cotton-like	Dark green

c. Results of Microscopic Characteristics and Biochemical Properties

The microscopic characteristics of the MB1 isolate revealed rod-shaped, gram-negative bacteria, whereas the MB2 and MB4 isolates revealed rod-shaped, gram-positive bacteria. In terms of biochemical properties, the three bacterial isolates tested positive in the catalase and citrate tests, and were uniformly negative in the Indole test. Only MB2 tested positive in the motility test, and in the TSIA test, only MB2 revealed negativity with no gas formation. Regarding the fungal isolates MJ1 and MJ2, their colouring characteristics were nearly identical, with the only difference found in the conidia. For MJ1, the conidia were scattered, whereas for MJ2, they were not. These characteristics are detailed in Table 3.

Table 3.
Results of Microscopic Characteristics and Biochemical Properties of the Isolates

No	Isolate Code	Microscopic		Biochemical Properties				
		Form	Coloring	Catalase	Citric	Motility	Indol	TSIA
1	MB1	Rod	Gram Negative	+	+	-	-	g (-), H_2S (+), M/M
2	MB2	Rod	G. Positive	+	+	+	-	g (-), H_2S (-), M/K
3	MB4	Rod	G. Positive	+	+	-	-	g (-), H_2S (+), M/M

No	Isolate Code	Coloring Characteristics	Staining Results
1	MJ1	• Conidia are round in shape • Conidia heads disperse • Presence of metulae • Vesicle • Fine and erect conidiophores • Non-septate hyphae	
2	MJ2	• Conidia are round in shape • Presence of metulae • Vesicles • Fine and erect conidiophores • Non-septate hyphae	

d. Results of Phytochemical Screening of the Extract from the Rhizomes of the Squirrel's Head Fern

Phytochemical screening of the extract from the rhizomes of Squirrel's Head Fern revealed the presence of six types of compounds, namely from the Flavonoid, Phenol, Alkaloid, Saponin, Tannin, and Triterpenoid groups. This information is detailed in Table 4 below:

Table 4.
Results of Phytochemical Screening of the Extract from the Rhizomes of the Squirrel's Head Fern

No	Compound Name	Reactor	Test results	Information
1	Flavonoids	MG + HCL	+	when an orange color formed
	Phenol	FeCl3	+	when a bluish-green or dark blue color formed.
3	Alkaloids	HCL 2N + Dragendruff	+	when a yellowish-white precipitate formed.
4	Saponin	H2O	+	when foam formed
5	Tannin	F2Cl3 1%	+	when a bluish color
6	Triterpenoids	Anhydrous Acetic Acid + H2SO4	+	when a purple-brown

2. Discussion

There were five isolates successfully obtained, comprising three bacterial isolates and two fungal isolates. These isolates exhibited growth on Natrium Agar and Potato Dextrose Agar, fundamental media for bacterial and fungal cultivation, respectively. The isolates were coded as

MB1, MB2, MB4, MJ1, and MJ2. Bacterial identification followed the guidelines outlined in Bergey's Manual of Determinative Bacteriology Seventh Edition (Breed et al., 1957), while fungal identification followed the criteria provided in the Description of Medical Fungi (Ellis et al., 2007) and Suryani et al. (2020).

Macroscopic, microscopic, and biochemical properties analyses of the MB1 isolate revealed its affiliation with the genus Streptobacillus. This determination was based on observed colony characteristics, including a round shape, flat edges, a convex surface, and a creamy white colour. Microscopically, Streptobacillus cells exhibited a rod-like shape, were gram-negative, non-fermentative for glucose, non-motile, produced H2S, and utilised citrate as a carbon source. These characteristics aligned with Bergey's Manual and were supported by Zulfarini et al. (2022). The genus Streptobacillus, identified as a proteolytic bacteria, exhibited the ability to produce protease enzymes, as affirmed by Pamaya et al. (2018). These enzymes possess hydrolytic capabilities, breaking peptide bonds in proteins into oligopeptides and amino acids. Notably, these proteases find applications in diverse industries such as pharmaceuticals, leather, and food. Endophytic bacteria-derived proteases are recognized as some of the most potent enzymes in this category.

The MB2 isolate revealed its affiliation with the genus Bacillus based on its observed characteristics, including a round shape, flat edges, a convex surface, and a white colour. It was a gram-positive bacterium with a rod-shaped cell, capable of fermenting glucose, exhibiting bacterial motility, utilising citrate, and being non-motile. Puspita et al. (2017), who isolated and characterised endophytic Bacillus bacteria from oil palm plants (Elaeis guineensis jacq), similarly described macroscopic characteristics of a round shape, flat edges, a convex surface, and white colony colour. These bacteria were noted to produce the catalase enzyme and exhibit motility. Bacillus, as an endophytic bacteria in plants, is recognized for its potential to produce antibacterial compounds, as outlined by Zulfarina (2022). Bacillus bacteria are known to generate secondary metabolite compounds with significant agricultural benefits.

The MB4 isolate revealed its affiliation with the genus Corynebacterium based on its observed characteristics, including irregular shape, flat edges, a convex surface, and a cream colour. It was capable of producing the catalase enzyme and was non-indole. Susanti (2021) and Susilowati et al. (2018) successfully isolated bacteria of this genus with identical characteristics. Corynebacterium is acknowledged for its ability to control pests and diseases in plants. Nuryani et al. (2018) explained the potential use of Corynebacterium bacteria as a biofungicide to manage

white rust disease and promote the growth of chrysanthemum (Chrysanthemum) plants. Additionally, Ismail et al. (2011) asserted that Corynebacterium bacteria can effectively control leaf blight in rice plants.

The MJ1 and MJ2 isolates exhibited nearly identical characteristics and revealed their affiliation with the genus Aspergillus. The only discernible differences lay in the colour and shape of the colonies, suggesting the possibility of these being two distinct Aspergillus species. According to the Description of Medical Fungi (2007), colonies of Aspergillus are initially round, flat, and white but quickly transition to a dark greenish-white hue with age. Conidial heads typically radiate and then divide into loose columnar forms, mostly biseriate, but with some heads directly borne on vesicles (uniseriate). Conidiophore stipes are hyaline and rough, often more visible near the vesicle. The round-shaped conidia are characteristic of Aspergillus flavus, a finding supported by Fitria and Setiawati (2020). Meanwhile, the MJ2 isolate exhibited the exact characteristics of A. fumigatus, as evidenced by research conducted by Nontji et al. (2023).

Phytochemical screening of the extract from the rhizomes of the Squirrel's Head Fern indicated the presence of five types of compounds: Flavonoids, Phenols, Alkaloids, Saponins, Tannins, and Triterpenoids. This suggests that the rhizomes of the Squirrel's Head Fern possess antimicrobial and antioxidant activity.

CONCLUSION

There were five isolates identified in the rhizomes of the Squirrel's Head Fern, namely MB1 belonging to the genus Streptobacillus, MB2 belonging to the genus Bacillus, MB4 belonging to the genus Corynebacterium, and MJ1 and MJ2 belonging to the genus Aspergillus. The results of the phytochemical screening indicated the presence of six positive compounds in the rhizome, namely flavonoids, phenols, alkaloids, saponins, tannins, and triterpenoids.

Suggestion

Molecular identification, including sequencing, is imperative to confirm the species of each isolate. Additionally, testing for antimicrobial activity against various pathogenic bacteria should be conducted.

REFERENCES

Chatterjee, S.; Bhattacharya, S.; Choudhury, P. R.; Rahaman, A.; Sarkar, A.; Talukdar, A. D.; Mandal, D. P.; Bhattacharjee, S. : Drynaria quercifolia suppresses paracetamol-induced hepatotoxicity in mice by inducing Nrf-2. Source: Bratislava Medical Journal / Bratislavské Lekárske Listy . 2022, Vol. 123 Issue 2, p110-119. 10p.

Ellis, D. H., Davis, S., Alexiou, H., Handke, R., & Bartley, R, 2007. Descriptions of medical fungi (Vol. 2). Adelaide: University of Adelaide.

Febriasyah, Mustofa, Triana H. dan Jaka Widada., 2021. Isolasi Senyawa Aktif Dari Streptomyces Sp. Gmy01 Dan Uji Sitotoksik Pada Sel Kanker Payudara Secara In Vitro Dan In Silico

Fitria, N., & Setiawati, F., 2020. Modifikasi Media Jagung (Zea mays) dan Kacang Tanah (Arachis hypogea) sebagai Media Pertumbuhan Aspergillus flavus.Jurnal Reka Lingkungan, 8(1), 57-66.

In Vitro Evaluation Of Antidiabetic And Cytotoxicity Potentials Of The Rhizome Extract Of Drynaria Quercifolia (L.) J. Smith Prasanna G*, Devi R, Ishwarya G. 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons. org/licenses/by/4. 0/) DOI: https://dx.doi.org/10.22159/ajpcr.2019.v12i11.35078R

Ismail, N., Luice, A., Taulu dan Bahtiar., 2011. Potensi Corynebacterium sebagai pengendalian penyakit hawar daun bakteri pada tanaman padi. Seminar Nasional Serealia. Hal: 459-465. Balai Pengkajian Teknologi Pertanian: Sulawesi Utara

Nontji, M., Palad, M., Diniarti, W., Saidah, S., & Aminah, A., 2023. Isolasi dan Inventarisasi Cendawan Endofit pada Tanaman Tomat. AGRIUM: Jurnal Ilmu Pertanian, 26 (1).

Nuryani, W., Silvia, E., Hanudin dan Kurniawan, B., 2018. Aplikasi biofungisida berbahan aktif Corynebacterium sp ramah lingkugan dalam pengendalian penyakit karat putih pada krisan. Jurnal Teknologi Lingkungan, (9). 1: 23- 32

Pamaya, D., Muchlissin, S. I., Maharani, E. T. W., Darmawati, S., & Ethica, S. N., 2018. Isolasi Bakteri Penghasil Enzim Protease Bacillus Amyloliquefaciens Irod2 Pada Oncom Merah Pasca Fermentasi 48 Jam. In Prosiding Seminar Nasional & Internasional 1(1).

Phytochemical screening and GC-MS Analysis of Rhizome of Drynaria quercifolia Mr. MK. Nithin*, Dr. G.Veeramani, Dr. S. Sivakrishnan. ISSN 0974-3618 (Print) www.rjptonline.org) Research J. Pharm. and Tech. 13(5): May 2020

Puspita, F., Ali, M., & Pratama, R., 2017. Isolasi dan karakterisasi morfologi dan fisiologi bakteri Bacillus sp. endofitik dari tanaman kelapa sawit (Elaeis guineensis Jacq.). Jurnal Agroteknologi Tropika, 6(2), 44-49.

Suryani, Y. (2020). Mikologi.

Susanti, A. R. E. H., 2021. Isolasi dan karakterisasi bakteri endofit dari tanaman aloe vera sebagai penghasil senyawa antibakteri terhadap propionibacterium acnes dan staphylococcus aureus. Universitas Islam Negeri Maulana Malik Ibrahim).

Susilowati, D.N., Hendra, G., Erny, Y., Mamik, S dan Ika R., 2018. Karakterisasi bakteri endofit tanaman purwoceng sebagai penghasil senyawa steroid dan antipatogen. Jurnal Littr, (24). 1: 1-10.

Zulfarina, Z., Rosiana, Y., Ayudia, D., & Darmawati, D., 2022. Isolasi Bakteri Endofit dari Tanaman Laban (Vitex Pubescens Vahl) sebagai Antibakteri. JST (Jurnal Sains dan Teknologi), 11(1), 85-92.