

Endophytic Mycoflora from Mace and Seed of Nutmeg (*Myristica fragrans* Houtt) as a Source of Antifungal Compounds

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ABSTRACT

The nutmeg extract had been recognized as the source of antioxidant and antimicrobial activity. The aims of this study were to obtain endophytic fungi isolated from the mace and seed of Nutmeg (*Myristica fragrans* Houtt) and to determine antifungal activity of those isolates. Microbiol routine assay were conducted and it consists of three stages, namely isolation of endophytic fungi, identification of fungus and antifungal activity test by using dual culture. Sixteen endophytic fungus isolates were found in this study. Those are identified as *Alternaria* sp (BPB 1.3), *Aspergillus* sp (BPB 2.2, BPB 2.3, BPB 2.4) and sterile hyphae (FPB 1.1, FPB 2.2, FPB 3.3, FPB 3.4, FPB 4.3, FPB 5.2, FPB 5.3, BPB 3.1, BPB 3.2, BPB 5.1, BPB 5.2, and BPB 5.4), respectively. The isolate from Mace organ (FPB 2.2) had the highest antifungal activity, 51.67% while FPB 1.1 isolate had the lowest one (5%). The BPB 3.2 isolate had the highest antifungal activity in seed organ while BPB 5.2 isolate had the lowest ones, respectively 63,48% and 12,5%. Endophytic fungi which isolated from mace and seed of nutmeg (*M. fragrans* Houtt) are the potential to be used as an antifungal.

Keywords: *antifungal activity, endophytic fungi, mace and seed, nutmeg*

1. INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt) was known as a spice crop that are potentially in term of antioxidant, anti-inflammatory, hepatoprotective, chemoprotective agent, and antimicrobe sources. Nutmegs are native to Banda island, then it spread out to other islands in the eastern part of Indonesia including North Maluku, Papua and Sulawesi [1]. The essential part of this plant are maces and seeds which economically profitable. Reported that mace and seed of nutmeg are the sources of antioxidant agents. Mace and seed are also being the main source of important compounds such as myristicin, saphrole, elemicin and isoeugenol [3].

Many studies has been reporting over the potential of nutmeg referring to its bioresources properties like antioxidants, anti microbes and antifungal [4]. Due to other bioresources properties of nutmeg, here we specifically on endophytic microflora. Endophytic is the group among microorganism which consisting of bacteria and fungi, where its life cycle inhabit is mostly found in the tissue of normal plant that surprisingly it does not harm its host plant and/or does not give any symptoms at all. Therefore, in this study, we carry out the isolation of endophyte mycoflora from mace and seed of nutmeg (*M. fragrans* Houtt) and determination antifungal activity of those isolates against pathogen.

2. METHODS

Sample of nutmeg fruits were collecting from public/common plantation/farming which located at Ternate. Aseptically, nutmeg fruit were cut and transfer into sterile bag before Microbiol treatment. Microbiol assay was carry out in three-step which is isolation the endophyte mycoflora by direct culture, morphology identification and antifungal activity by using dual culture. Surface sterilization.

Mace and seed are separated carefully from the nutmeg fruit. Both of it, are then washing for 2 up 5 minutes gently with water before surface sterilization treatment. Aseptically, mace and seed are cutting into small size. Every pieces are soaking in seventy percent alcohol for 3 minutes, one percent sodium hypochlorite for 5 minutes. Before each pieces are soaking again with seventy percent alcohol for 0,5 minutes, every drop of those ex-solution was dried with tissue paper then final step gently wash with sterile aquadest for 1 minute, respectively. This step was repeated three times, Samples are cutting in 1 cm x 1 cm size.

Isolation the endophyte

Into each media of Potato Dextrose Agar (PDA) enriched by mace extract and PDA enriched by seed extract, every pieces of mace and seed with certain size were put in the surface of the media, aseptically. Each plate contain four pieces of mace and seed, separately. All plate are incubate

at 28 – 30 °C for one until two weeks before pure culture are conducted. Morphology identification of endophytic fungi was by using illustrated genera imperfect fungi [5] and Intoduction of tropical fungi [6]. Pure culture are then used for anthagonist assay.

Anthagonistic assay

Dual culture metode are implementong in this assay. Pathogen *Fusarium oxysporum* are plate on *Potato Dextrose Agar* (PDA) and incubate for one week, separately. Both of the isolate of endophytic fungi and *F. oxysporum* are plate together where endophytic fungi and the pathogen are put as counterpart as shown in fig.1. Antifungal compound was observed by zone of growth barruer between pathogen against endophytic fungus. Barrier zone of fungal growth can be count by using the formula refer to Alfizar *et al.* (2013):

$$P = \frac{r1 - r2}{r1} \times 100\%$$

Which

- P = coefficient of zone of growth barrier of pathogen against endophytic fungi which is measures in percentage (%)
- r1 = the distance of growing pathogen that avoid from growing endophytic which is measures from the central point of growing pathogen
- r2 = = the distance of growing pathogen that close to growing endophytic which is measures from the central point of growing pathogen

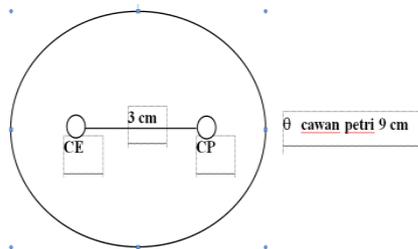


Figure 1. Dual culture technique are use to assay antifungal activity of endophytic mycoflora from nutmeg

III. RESULTS AND DISCUSSION

Sixteen endophytic mycoflora was isolate in our study as describe on table 1. Growing hyphae was emerge from both mace and seed pit of nutmeg (*M. fragrans* Houutt) on the surface of media. Potato dextrose Agar which is added with mace and seed extract on every plate, respectively. Six isolates origin from mace and nine isolates from seed. As shown below on fig.2, the colony of growing hyphae origin from mace and seed, respectively on plate A and B.

Comparing to our study, about fifteen endophytic fungus have been succeed isolated from nutmeg [7]. Another report revealed that endophytic fungi can be different in species or genus which is isolate from one part to another in one plant [8]. Our finding shows all endophytic fungi origin from mace have no reproductive structure/stage in its hyphae (sterile hyphae). Same result have been reported

in *Theobroma cacao* [9]. Nine isolates of endophytic fungi origin from seed are belong to genus *Aspergillus*, *Aternaria* and sterile hyphae, respectively.

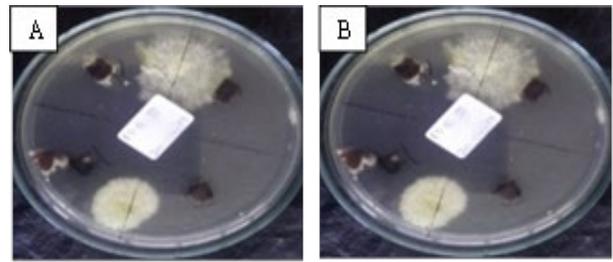


Figure 2. Colony of growing hyphae of endophytic mycoflora from mace (A) and seed (B) of nutmeg

Table 1. Endophytic Mycoflora origin from mace and seed of nutmeg (*Myristica fragrans* Houut)

Isolate	source	Level of taxa	Identification result
BPB 1.3	seed	Genus	<i>Alternaria</i>
BPB 2.2	seed	Genus	<i>Aspergillus</i>
BPB 2.3	seed	Genus	<i>Aspergillus</i>
BPB 2.4	seed	Genus	<i>Aspergillus</i>
BPB 3.1	seed		Steril hyphae*
BPB 3.2	seed		Steril hyphae
BPB 5.1	seed		Steril hyphae
BPB 5.2	seed		Steril hyphae
BPB 5.4	seed		Steril hyphae
FPB 1.1	mace		Steril hyphae
FPB 2.2	mace		Steril hyphae
FPB 3.3	mace		Steril hyphae
FPB 4.3	mace		Steril hyphae
FPB 5.2	mace		Steril hyphae
FPB 5.3	mace		Steril hyphae

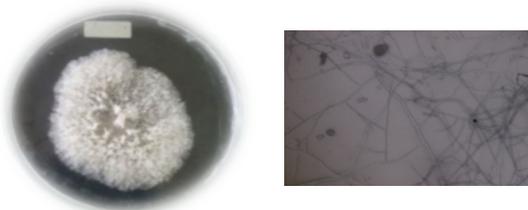


Figure 3. Isolate of BPB 3.2 from seed of nutmeg where has highest antifungal activity with score 63,48 %

Antifungal activity was conducting by *dual culture technique*. The result shows that all the isolates having antifungal activity as describe on figure 4 and 5 below.

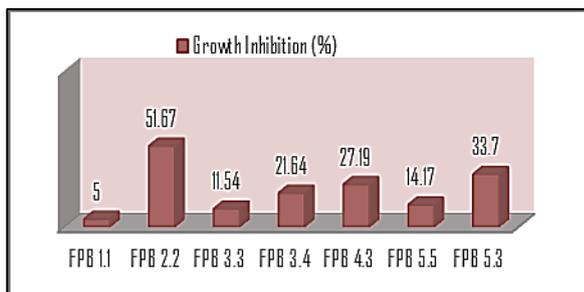


Figure 4. Percentage of growth inhibition of endophytic fungi isolated from nutmeg mace against pathogen *F. Oxysporum*

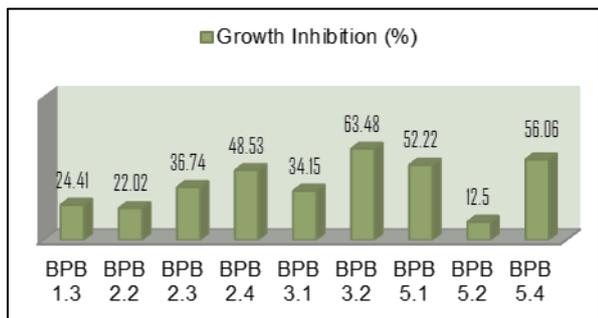


Figure 5. Percentage of growth inhibition of endophytic fungi isolated from nutmeg seed against pathogen *F. oxysporum*

According to the data of antifungal activity from mace and seed, FPB 2.2 dan BPB 3.2 isolates are the promising candidate for further investigate as source of antifungal compound. Those isolate given the highest inhibition against pathogen *F. oxysporum*. Interestingly, both of isolates are sterile hyphae. While the lowest score came from FPB 1.1 and BPB 5.2 isolates. Some investigation about endophytic fungi as antimicrobe especially come from group of sterile hyphae have been reporting by [10,11]. Endophytic fungi may produce enzyme as antifungal source therefore they can suppress growth of pathogen. Mace and seed of nutmeg have many active compound especially myristicin. Rather seed than mace, our investigation report that antifungal activity of endophytic fungi isolated from seed (Figure 3) more

strong than endophytic fungi isolated from mace. The main compounds of mace and seed of nutmeg are belong to group of terpene secondary metabolites such as myristicin (both), terpineol-4, safrole, palmitin, dodecanoic acid, ethenyl ester (compounds in mace), hexadecanoic acid, 2,3- dihydroxypropyl ester, dodecanoic methyl ester, methyl palmitate within vary in its content [4]. It can be reveal that in seed component mostly contain of larger group of fatty acids rather than mace. Refer to its chemical composition, mace and seed are possibly as source of nutrition for endophytic fungi who lives within tissue of nutmeg plant. How endophytic fungi metabolize it is still remain unclear but the possible clue was a positive correlation (interaction) between nutmeg plant (host) and endophytic mycoflora as mutualism association.

Antimicrobe activity of any part of nutmeg plant also have been reported. Endophytic fungi might be share the same role as a plant protection from inner tissue as well as plant itself which is it can produce antifungal or antibacterial activity through its secondary metabolite.

IV. CONCLUSION

Based on the research result, this study revealed that sixteen endophytic mycoflora origin from mace and seed of nutmeg (*Myristica fragrans* Houtt) was successfully isolated. Steril Hyphae are dominant isolates which is consist of twelve isolates, genus *Aspergillus* consist of three isolates and one isolates belong to genus *Alternaria*. The highest antifungal activity was rising from BPB 3.2 isolated from nutmeg seed with 63,48% of growth inhibition and the lowest one comes from FPB 1.1 isolated from nutmeg mace. There are two isolates as a promising candidate as a source of antifungal compounds from both mace and seed nutmeg, they are BPB 3.2 and FPB 2.2 isolates.

ACKNOWLEDGMENT

This research was a part of student bachelor degree with collaborate our institution research grand. We thank to Universitas Khairun and Biology Education laboratory to support the facilities.

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