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Research Article

Bioconversion of steroid compounds due to interaction of the bark extract of *Michelia champaca* with fungi *Curvularia verruculosa*

I Gusti Agung Gede Bawa*
Chemistry Department, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia
Sri Rahayu Santi
Chemistry Department, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia
I Made Sukadana
Chemistry Department, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia
Anak Agung Bawa Putra
Chemistry Department, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia

*Corresponding author. Email: gede_bawa@unud.ac.id

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Abstract
Functional groups bioconversion of steroid compounds was observed in the interaction of secondary metabolites of *Michelia champaca* with *Curvularia verruculosa* fungal. In an inhibited mechanism, this extract can lyse the cell walls of the fungus. This research aims to determine changes in secondary metabolite compounds involved in the inhibition process of *M. champaca* bark extract on the growth of the fungus *C. verruculosa*.The interaction of secondary metabolites of *M. champaca* with *C. verruculosa* fungi was observed by biomass test method and bioconversion analysis of steroid compounds was observed by gas chromatography-mass spectroscopy (GC-MS). Based on the biomass test of *M. champaca* bark extract with 1% concentration can inhibit the biomass formation of *C. verruculosa* fungal with inhibiting power is 74.73% and at 2% concentration has been able to inhibit completely the biomass formation of *C. verruculosa* fungal (100%) for a-8 days period of incubation. The bioconversion of the functional groups in steroid compounds was observed by changing allopregnane-7α, 11α-diol-3,20-dione compounds to pregnane-3,20-dione; pregnane-3,11-diol-20-one; and pregnenolone, as well as the bioconversion of β-sitosterol compounds to pseudosarsapogenin-5,20-dien; (3β, 24S)-stigmastane-5-en-3-ol; and (3β, 22E)-stigmastane-5,22-dien- 3-ol.

Keywords : Bioconversion, *Curvularia verruculosa*, extract of *Michelia champaca,* interaction, steroid compounds

INTRODUCTION

Curvularia verruculosa fungi can cause leaf spot disease in rice plants (Bawa, 2019). The control of this disease using botanical pesticides is being developed. The *Michelia champaca* bark extract can potentially develop into a botanical pesticide. This extract can inhibit the growth of *C. verruculosa* fungal with very strong inhibitory power (Bawa, 2021). Various mechanisms of plant extracts' inhibition of fungal cell growth have been reported. One of them, namely plant extracts, is able to lyse fungal cell walls (Otang *et al*., 2011; Hashem *et al*., 2016). Damage to the drying cell walls is caused by biochemical changes in the components of the composing compounds of fungal cells, especially the steroid compounds. Steroids are compounds that are very abundant in nature, and have various physiological activities. These compounds include androstane, estrane, gonane, cholestane, and protostane (Dembitsky, 2023). These compounds are found in insects (Masterson *et al*., 2022), plants (Yerlikaya *et al*., 2023), vertebrates (Doyle and Meeks, 2018), and fungi (Dias *et al*., 2019). In fungi, steroid compounds are found in the fungal cell membrane of the ergosterol type as the main component (Hu *et al*., 2017). Damage to fungal cell membranes due to interactions with active antifungal compounds also results in structural changes in ergosterol compounds. This change is the result

of the bioconversion or biotransformation process. These enzyme catalyzed reactions include hydroxylation, oxidation, dehydration and condensation, amination and deamination, dehydrogenation, decarboxylation and isomerization (Sameera, 2011). Biotransformation of steroid compounds is more prevalent in steroid hydroxylation, dehydrogenation, and side chain termination of sterols (Bhatti and Khera, 2012; Donova and Egorova, 2012). This reaction was also observed in the interaction of the *Curvularia verruculosa* fungi with the *Michelia champaca* bark extract. This research aims to determine changes in secondary metabolite compounds, especially in steroid compounds, that are involved in the inhibition process of *M. champaca* bark extract on the growth of the fungus *C. verruculosa*.

MATERIALS AND METHODS

Material

The bark of *Michelia champaca* was collected in Bongkasa Village, Badung regency, Bali-Indonesia. The *C. verruculosa* CIMAP Og 22018 strain was isolated from leaves of rice plants infected with leaf spot disease in Subak Ayunan, Ayunan Village, Abiansemal, Badung Bali and it was deposited in Biopesticide Laboratory Udayana University.

Methodology

Biomass test method

The inhibitory activity of the *Michelia champaca* bark extract against the growth of fungi *C. verruculosa* was tested using the biomass test method (Rendowaty *et al*., 2017) with some modification. This test was carried out by varying the extract concentration, namely 0% as a control fungus (C_1); 1.0% (T_1); 2.0% (T_2) and 1.0% extract without fungi as a control extract (C_2). The test was carried out by growing the suspension of *C. verruculosa* on PDB medium in 250 ml Erlenmeyer volume. The bark extract of *Michelia champaca* was put into Erlenmeyer according to the concentration tested, and then 1 ml of the fungal suspension is added into it, except for C_2. The final culture volume (PDB medium + extract + fungal suspension) was 100 ml. These cultures were incubated in the dark at room temperature for 8 days. Fungal biomass harvesting was carried out by filtering the formed biomass deposits using glass filter paper. The filtrate obtained was extracted successively with 3 x 50 ml ethylacetate. The extract obtained was evaporated by the solvent with a rotary vacuum evaporator, so that four (4) ethylacetate extracts were obtained, namely extracts of C_1, T_1, T_2, and C_2. Each of these extracts was analyzed by GC-MS.

Composition analysis of secondary metabolite compounds by gas chromatography-mass spectroscopy

Fungal biomass harvesting was carried out by filtering the formed biomass deposits using glass filter paper. The precipitate obtained was dried at 60^0 C until its weight was constant (Rendowaty *et al*., 2017). The filtrate obtained were extracted successively with 3 x 50 ml ethylacetate. The extract obtained was evaporated by the solvent with a rotary vacuum evaporator, so that four (4) ethylacetate extracts were obtained, namely extracts of C_1, T_1, T_2, and C_2; Each of these extracts was analyzed by GC-MS.

RESULTS AND DISCUSSION

Based on the biomass test of the bark extract of *Michelia champaca* with 1% concentration can inhibit the biomass formation of *C. verruculosa* fungal with inhibiting capability is 74.73% and at 2% concentration has been able to inhibit completely the biomass formation of *C. verruculosa* fungal (100%) for a-8 days period of incubation (Table 1). This result is better than the result of the study conducted by Bajwa *et al.* (2003) found out that the water extract of *Parthenium hysterophorus* at

Table 1. The result of the inhibition of fungal growth using the biomass test method

Treatment (extract concentration) (%)	Test	Fungal Biomass (g)	Inhibition Power (%)	Average of Inhibition Power (%)
C1 (0%) (Fungal Control)	1	0.1954	0.00	0.00
	2	0.2017	0.00	
	3	0.1997	0.00	
	4	0.1760	0.00	
T1 (1%)	1	0.0518	73.49	74.73
	2	0.0436	78.38	
	3	0.0503	74.81	
	4	0.0489	72.22	
T2 (2%)	1	0.0000	100.00	100.00
	2	0.0000	100.00	
	3	0.0000	100.00	
	4	0.0000	100.00	

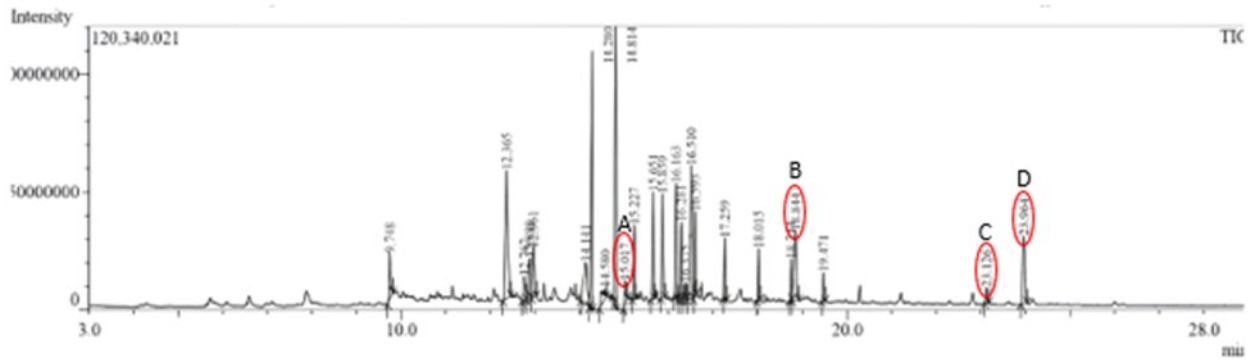

Fig.1. *Spectrum GC-MS T_1 (1%): A (Allopregnan-7a,11a-diol-3,20-dion); B (Stigmastane-5-en-3-on (3b,24S); C (Stigmastane-5,22-dien-3-on (3b,22E); D (b-sitosterol)*

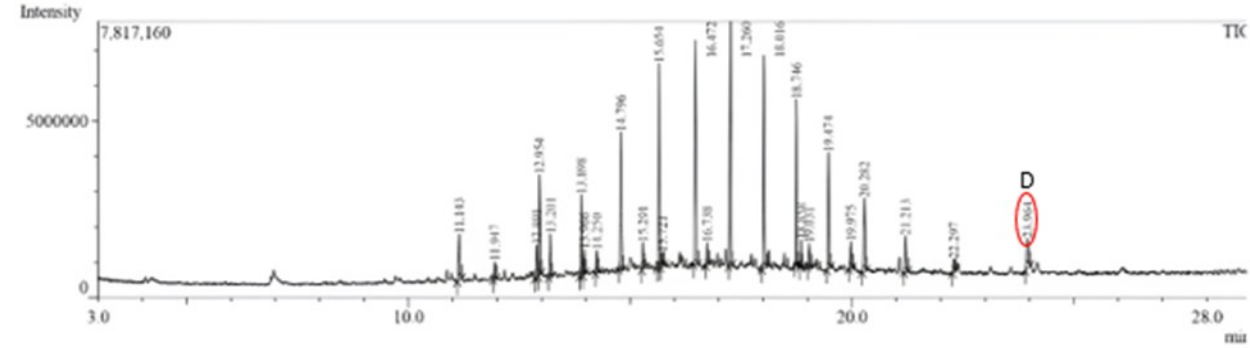

Fig. 2. *Spectrum GC-MS C_1 (control fungi): D (b-sitosterol)*

5% concentration has been able to reduce the biomass formation of the *Aspergillus niger* fungal by 29% for a-5 days period of incubation, while the biomass formation of *Drechslera tetramera* fungal was reduced by 36% at 30% concentration for a-15 days period of incubation. However, this result is uglier than Zargar (2014) found out that *Rhazya stricta* Decne extract at 10 mg/ml concentration was been able to inhibit the biomass formation of *Penicillium notatum* and *Aspergillus niger* fungal by 91.8 and 93.2%, respectively, for a-15 days period of incubation.

The results of GC-MS analysis showed that functional group bioconversion had occurred against steroid compounds in the fungal cell membrane (C_1) and the *Michelia champaca* bark ekstrak C_2). The presence of (3β, 24S)-stigmastane-5-en-3-ol (code B) and (3β, 22E) -stigmastane-5,22-dien-3-ol (code C) steroid compounds in T_1 treatment (Fig. 1) was thought to be due to the biotransformation of β-sitosterol compounds (code D) in the control fungus (C_1) (Fig. 2). The compound of (3β, 24S)-stigmastane-5-en-3-ol and β-sitosterol compound differ only in the space arrangement of the groups attached to C24. The presence of the enzyme ERG5 (sterol C-22 desaturase) was able to convert β-sitosterol to stigmastane-5,22-dien-3-ol (3β, 22E). This is based on the statement of Kristan and Rizner (2012) and strengthened by Long & Zhong (2022), where the presence of the C-22 desaturase enzyme was able to change ergosta-5,7,24-triene-3β-ol to ergosta-5,7,22,24-tetraene-3β-ol. The biosynthesis of (3β, 24S)-stigmastane-5-en-3-ol and (3β, 22E)-stigmastane-5,22-dien-3-ol compounds from β-sitosterol is presented in Fig. 3.

The presence of allopregnane-3β,7α-diol-20-one (code A) and pregnane-3,20-dione compounds (code E) in T_2 treatment (Fig. 4), was thought to be due to the biotransformation of pregn-5-ene-20-one, 3-hydroxy or pregnenolone compounds (code F) contained in the filtrate control of extract (C_2) (Fig. 5). The presence of 7α-hydroxylase and 5α-reductase enzymes can convert pregnenolone to allopregnane-3β,7α-diol-20-one, whereas the presence of the enzymes

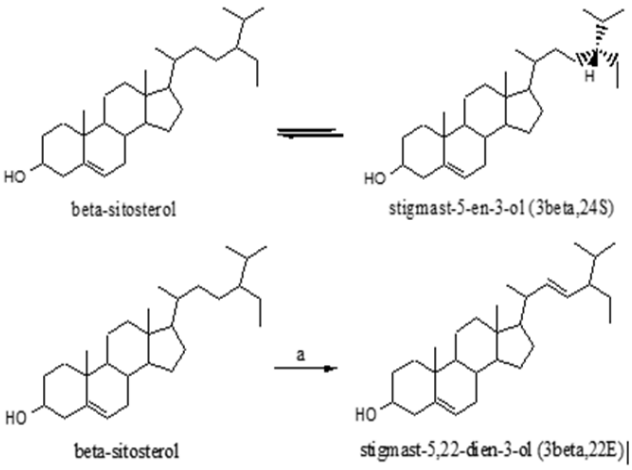

Fig. 3. *Biosynthesis of stigmastane-5-en-3-ol (3β, 24S) and stigmastane-5,22-dien-3-ol (3β, 22E) compounds from β-sitosterol (a: enzyme ERG5 (sterol C-22 desaturase)*

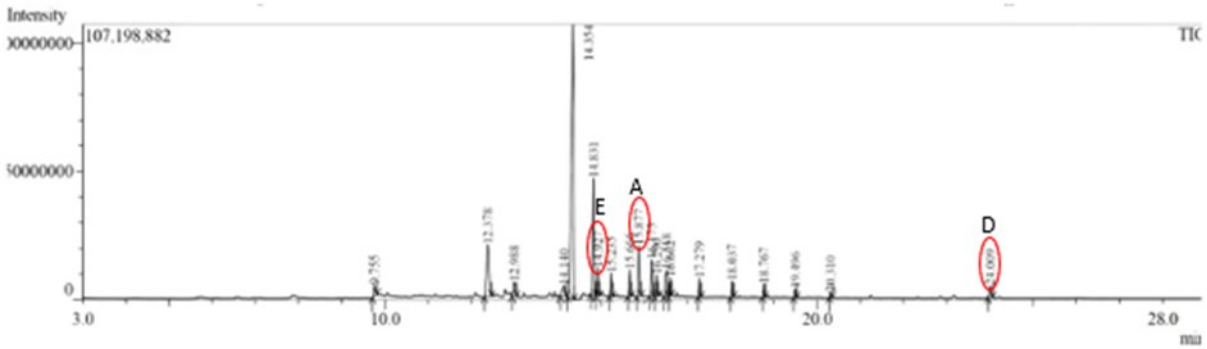

Fig. 4. *Spectrum GC-MS T_2 (2%): A (Allopregnane-3b,7a-diol-20-one); D (b-sitosterol); E (Pregnane-3,20-dione)*

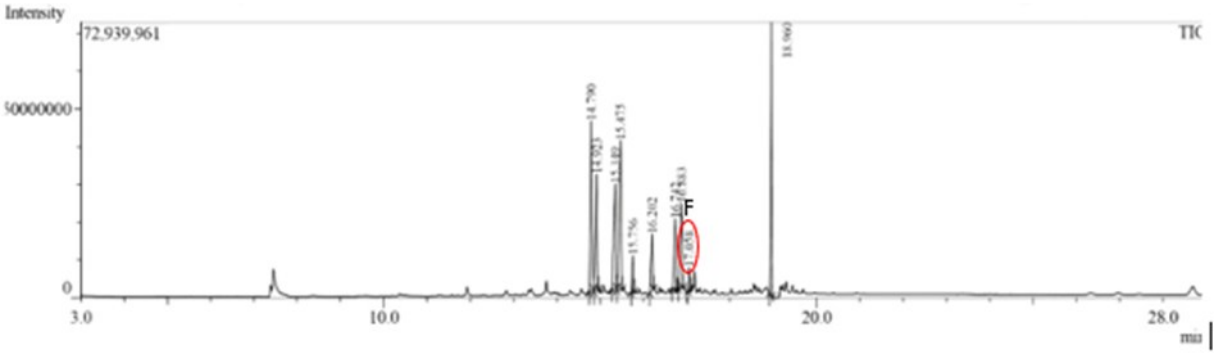

Fig. 5. *Spektrum GC-MS C_2 (control extract): F (Pregn-5-ene-20-one, 3-hydroxy) (Pregnenolone)*

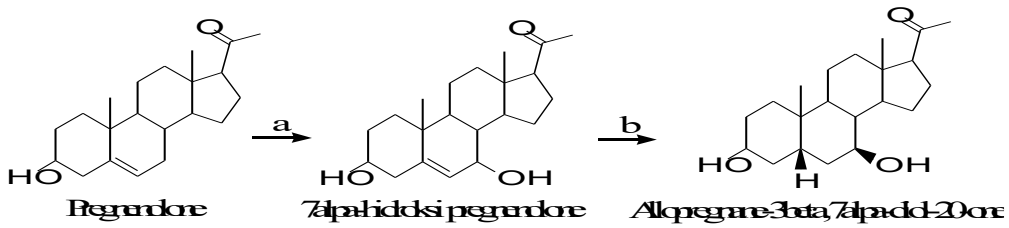

Fig. 6. *Biosynthesis of the allopregnane-3β, 7α-diol-20-one compound from pregnenolone (a: 7α-hydroxylase enzyme; b: 5α-reductase enzyme)*

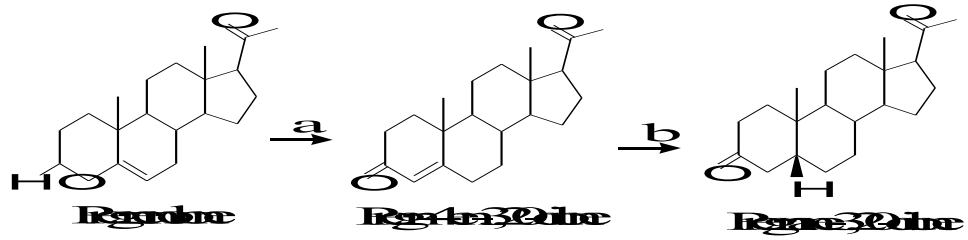

Fig. 7. *Biosynthesis of the compound pregnane-3,20-dione from pregnenolone (a: enzyme 3β-hydroxysteroid dehydrogenase; b: 5α-reductase)*

3β-hydroxysteroid dehydrogenase and 5α-reductase can convert pregnenolone to pregnane-3,20-dione (Kristan and Rizner, 2012; Kollweov *et al*., 2023). The 7α-hydroxylase enzyme converts pregnenolone to 7α-hydroxy pregnenolone. This result is supported by Lobastova *et al.* (2009) who reported the 7α-hydroxylase enzyme from *Gibberella zae* VKM F-2600 is able to convert dehydroepiandrosterone (DHEA) to 7α-hydroxy-DHEA. The 5α-reductase enzyme can convert 7α-hydroxy pregnenolone to allopregnane-3β, 7α-diol-20-one. This result is supported by Holland *et al*. (1994), which stated the 5α-reductase enzyme found in several *Penicillium* spp. (*P. chrysogenum, P. decumbens,* and *P. crustoum*) can reduce the double bond in testosterone to 5α-dihydrotestosterone (5α-DHT). The biosynthetic reaction of the allopregnane-3β,7α-diol -20-one compound from pregnenolone is presented in Fig. 6.

The presence of 3β-hydroxysteroid dehydrogenase enzyme can convert pregnenolone to pregn-4-en-3,20-

dione. This result is supported by Simard *et al*. (2005) who reported the 3β-hydroxysteroid dehydrogenase enzyme was able to convert 5-en-3β-hydroxysteroid such as pregnenolone to pregn-4-en-3,20-dione. The activity of the 3β-hydroxysteroid dehydrogenase enzyme was detected in *Aspergillus tamari,* which is able to convert 5-en-steroids such as DHEA and 3β,17β-dihydroxyandrost-5-ene into their 3-ketosteroid form (Hunter *et al*., 2009). The presence of the 5α-reductase enzyme can convert pregn-4-en-3,20-dione to pregn-3,20-dione. This is based on the statement of Kristan and Rizner (2012), which states that the 5α-reductase enzyme can convert androstenedione into androstanedione. The biosynthetic reaction of the compound pregnane-3,20-dione from pregnenolone is presented in Fig. 7.

Conclusion

In the present study, the *Michelia champaca* bark extract can inhibit completely the biomass formation of C. verruculosa fungal (100%) for a-8 days period of incubation at 2% concentration. At the molecular level, the reduced biomass formation was marked by the occurrence of biotransformation of the type steroid compound the occurrence of biotransformation of allopregnane-7α,11α-diol-3,20-dione type steroid compounds to pregnane-3,20-dione compounds; pregnane-3,11-diol-20-one; and pregnenolone, as well as the biotransformation of β-sitosterol compounds into pseudosarapogenin-5,20-dien, (3β,24S)-stigmastane-5-en-3-ol, and (3β,22E)-stigmastane-5,22-dien-3-ol compounds. Biotransformation of this compound was first discovered in the *C. verruculosa* fungal.

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Conflict of interest

The authors declare that they have no conflict of interest.

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