Research Article

Preliminary test of utilization of *Aspergillus niger* in the biotransformation of geraniol and its identification

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ABSTRACT

Background: Essential oils (EOs) have gained new interest in several aspects. As a natural product, EOs have attractive physicochemical characteristics with high added values that are environmentally friendly. EO also has diverse and relevant biological activities. A number of studies have highlighted the antimicrobial effects of EO even on multiresistant bacteria. Due to the complex chemical composition of EO, EO has a broad spectrum of biological and antimicrobial activity (antibacterial, antifungal, antiviral, pest control, and insect repellent). *Cymbopogon nardus* (L.) Rendle is one of the plants that contain EOs, usually known as citronella. citronella or *C. nardus* (L.) Rendle is one of the Cymbopogon species with quintessence oil which is widely used in the production of citronella, geraniol, citronellol, food, beverages, fragrances, soaps, body care products, and pharmaceutical products. The biotransformation study of geraniol, nerol, and citral by Aspergillus niger have been reported that the main bioconversion products obtained from geraniol and nerol by liquid culture of A. niger are linalool and α-terpineol. **Aim:** This study aims to utilize A. niger UICC 1012 for the biotransformation process of geraniol to obtain other compounds. **Result:** The results of identification with gas chromatography-mass spectroscopy showed that the biotransformation product of geraniol was linalool and alpha-terpineol. The value of RT and peak areas respectively; 12.657 and 145.009.110; 16.340 and 4.567.385 (day-1); 12.820 and 409.287.950; 16.422 and 35.681.840 (day-7). **Conclusion:** A. niger UICC 1012 can be used for biotransformation of geraniol to obtain linalool and alpha-terpineol.

KEY WORDS: *Aspergillus niger*, Biotransformation, Gas chromatography-Mass spectroscopy, Geraniol

INTRODUCTION

One of the essential oils (EOs) produced and used as a source of foreign exchange for Indonesia is fragrant citronella oil. According to data from the Ministry of Trade of Non-oil and Gas Exports for the period 2013–2018, the EOs export trend for 2013–2017 increased by 4% and changes in 2018–2017 were 19.85%.[1] Upstream industries in Indonesia are only able to provide EOs in rough forms that are directly exported to downstream industries such as the cosmetics industry, flavor, and fragrance, while industries that provide semi-finished materials that are needed by the downstream industry have not yet developed and have not utilized this potential to produce derivative products from EOs.

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There is a need to reduce the cost of natural products so that they are available to wider consumers. Biotechnology seeks to facilitate production and to reduce the market costs of natural EOs using various non-pollution methods. The most abundant method with better results is the biotransformation method catalyzed by microorganisms. Vanillin production from ferulic acid is in the range of 12–60 g/L using species *Amycolatopsis* and *Streptomyces*. Vanillin can be produced at 5 g/L by *Escherichia coli* and 37 g/L by *Saccharomyces cerevisiae*.[2]

Cymbopogon (*Poaceae*) is widely used as flavorings, fragrances, cosmetics, soaps, and detergents due to the distinctive aroma of lemon and rose-like. Constituents of EOs, *Cymbopogon,* are citral, geraniol, citronellol, and citronellal. The biological and pharmacological activities of these EOs have been growing rapidly in the past 10 years as anti-inflammatory, antibacterial, free radical decomposition, and other biological

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activities as well as important industrial applications, especially in food packaging.[3]

Biotransformation of metabolic compounds has been used to produce compounds that have high water solubility to allow for easier elimination of xenobiotics. Biotransformation of geraniol, nerol, and citral by *Aspergillus niger* produces linalool and α-terpineol. α-terpineol and linalool compounds have a higher economic value than nerol compounds.[4,5]

Based on the above research, the researchers want to investigate more about the use of *A. niger* in the biotransformation process of EOs.

MATERIALS AND METHODS

Materials

Geraniol (98.6%) was obtained from Shandong Nhu Pharmaceutical Co., Ltd, China. *A. niger* UICC 1012 was obtained from Indonesia of University [Figure 1].

Biotransformation Process

Biotransformation process of geraniol was performed by *A. niger* with the following steps:

- About 65 g Sabouraud Dextrose Agar (SDA) media is prepared in 1 L of distilled water in a Petri dish.
- *A. niger* is grown in SDA media.
- About 24 g of Potato Dextrose Broth (PDB) media is made in 1 L of distilled water.
- The inoculation of *A. niger* is made on PDB media.
- Biotransformation reaction was optimized, i.e., 50 ml of PDB media which had been inoculated with *A. niger* and incubated for 44 h and add 1 ml of geraniol. Incubated centrifugation at 350 rpm for 1–7 days.
- The biotransformation product was extracted with ethyl acetate (1:1), taken from the ethyl acetate phase, filtered with Whatman paper No. 1, and aerated the filtrate.

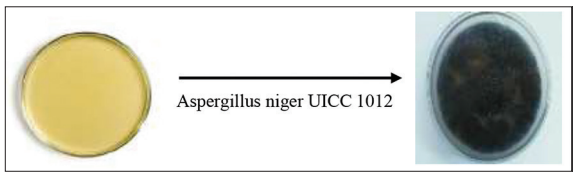

Figure 1: Preparation of *Aspergillus niger* UICC 1012

- Biotransformation products are identified by TLC and GC-MS.

RESULTS AND DISCUSSION

A. niger is a well-known fungus that has been used for many different biotransformations of organic compounds. *A. niger* produces several extracellular enzymes with significant industrial importance, including amylases, proteases, pectinases, lipases, and chitinases. *A. niger* also degrades celluloses and hemicelluloses and causes biodeterioration of oil-derived lubricants, polyvinyl chloride, and starch/polyethylene plastics. The ability of *A. niger* cultures to produce citric, oxalic, gluconic, fumaric, and gallic acids and vitamins, including biotin, thiamin, and riboflavin, is widely used in industry. *A. niger* has been one of the fungi used most frequently as a catalyst for the biotransformation of different organic compounds.[6]

Geraniol dilutes in methanol. Methanol is used as a solvent to accelerate biotransformation reactions because geraniol has very little solubility in water while methanol has a very high solubility in water, so geraniol dissolves perfectly and makes it dissolve in culture and facilitates interaction with fungus cells.[4]

Figure 2 shows the chromatogram of GC-MS of geraniol (SI = 94). Geraniol is a commercially important terpene alcohol occurring in the EOs of several aromatic plants. It is one of the most important molecules in the flavor and fragrance industries and is a common ingredient in consumer products produced by these industries. In addition to its pleasant odor, geraniol is known to exhibit insecticidal and repellent properties and used as a natural pest control agent exhibiting low toxicity. Geraniol has been suggested to represent a new class of chemoprevention agents for cancer. Other biological activities such as antimicrobial, antioxidant, and anti-inflammatory and some vascular effects have also been investigated. The effect of geraniol as a penetration enhancer for transdermal drug delivery has also attracted the attention of researchers and formulation scientists.[7] There are three sources to isolate this aroma chemical. Palmarosa oil, *Cymbopogon martinii*

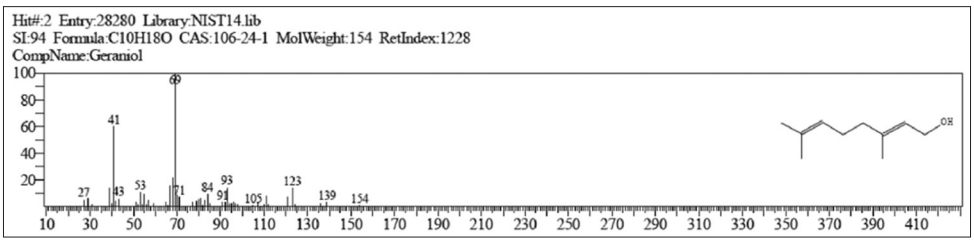

Figure 2: Chromatogram of gas chromatography-mass spectroscopy of geraniol

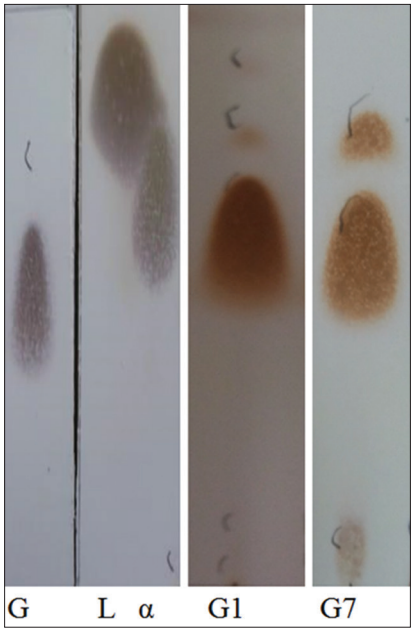

Figure 3: Chromatogram of Thin-Layer Chromathography of geraniol (G) and biotransformation product of geraniol (G1 and G7). G: Geraniol (standard), Rf = 0.5, L: Linalool (standard), Rf = 0.82, α: Alpha-terpineol (standard), Rf = 0.64, G1: Biotransformation product of geraniol at day 1, Rf = 0.5 and 0.8, G7: Biotransformation product of geraniol at day 7, Rf =0.5 and 0.8

commonly known "rosha" or russa, is the main source of geraniol (80–95%) and Jamrosa oil contains 80–89%. Another source of geraniol is *Cymbopogon winterianus* (Java citronella oil), which contains 40–45% including citronellol.[8] The biotransformation of geraniol, nerol, and citral by *A. niger* was studied. A comparison was made between submerged liquid, sporulated surface cultures, and spore suspensions. The main bioconversion products obtained from geraniol and nerol by liquid cultures of *A. niger* were linalool and α-terpineol.[5] Linalool was one of theminor compounds of *Cymbopogon*.[9] Linalool was components of many EOs. Linalool possesses several biological activities: anti-inflammation,[10] antibacterial, and antioxidant activity,[11] also for the fragrance industry and aromatherapy.[12]

The result of biotransformation of geraniol was analyzed using TLC with eluent, N-hexane: ethyl acetate, and sprayed with cerium sulfate stains as shown in Figure 3. Figure 3 shows that geraniol, linalool (standard). and alpha-terpineol (standard) and product of biotransformation of geraniol (G1 and G7) give the same spot with standard (G, L, and α) and other spots (other unkown compounds). This shows that geraniol had been biotransformation by *A. niger*.

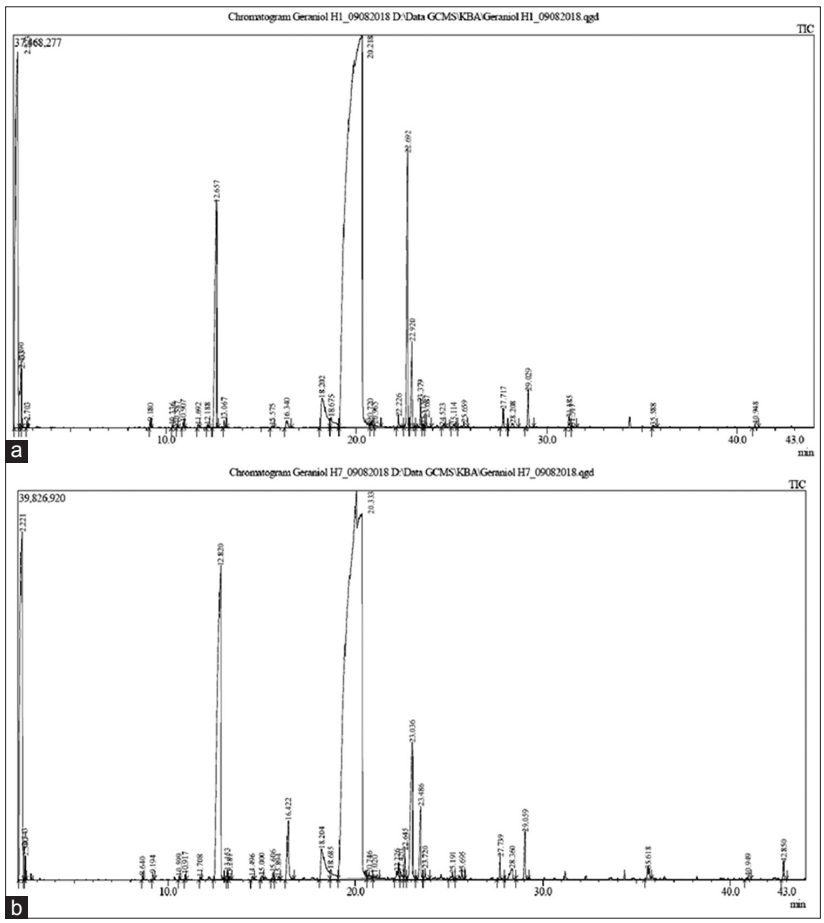

Figure 4: (a) Gas chromatography-mass spectroscopy (GS-MS) chromatogram biotransformation product of geraniol (day 1). (b) GS-MS chromatogram biotransformation products of geraniol (day 7)

Table 1: GC-MS analyses of biotransformation product of geraniol

Component	H1			H7		
	1 ml			1 ml		
	SI	RT	Area	SI	RT	Area
Alpha-terpineol	85	12.188	693.066	87	16.422	35.681.840
Linalool	96	12.657	145.009.110	95	12.820	409.287.950
Geraniol	94	20.218	2.020.436.979	95	20.333	2.121.024.235

GC-MS: Gas chromatography-mass spectroscopy

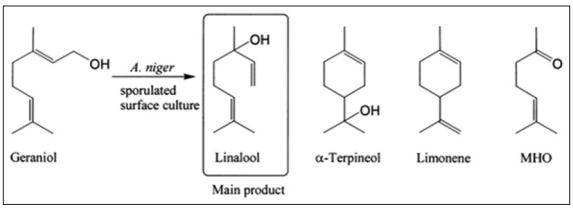

Figure 5: Biotransformation of geraniol by *Aspergillus niger*[5]

The results of biotransformamtion is confirmed by GC-MS as shown in Table 1 and Figure 4. From the result of analyses by GC-MS can be seen the component of linalool and alpha-terpineol well on day-1 or day-7. This proves that it has been biotransformation process as stated by previous researchers. It also appears that, on the 7th day, the area increased, and this shows that the resulting product is increased with the length of the biotransformation process.[5] The reaction of biotransformation sis was shown in Figure 5. Cultures of *A. niger* that adds hydroxyl, carbonyl, and other groups at specific positions or reduces double bonds have resulted in the production of valuable new compounds.[6]

CONCLUSION

Biotransformation process is occurred by *A. niger* UICC 1012 which produces linalool and α- terpineol.

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REFERENCES

1. Anonim. Available from: http://www.kemendag.go.id/id/economic-profile/indonesia-export-import/growth-of-non-oil-and-gas-export-commodity. [Last accessed on 2018 Oct 20].
2. Gounaris Y. Biotechnology for the production of essential oils, flavours and volatile isolates. A review. Flavour Fragr J 2010;25:367-86.
3. Ganjewala D. Cymbopogon essential oils : Chemical compositions and bioactivities. Int J Essent Oil Ther 2009;3:56-65.
4. Baser KH, Buchbauer G. Handbook of Essential Oils, Science, Technology and Applications. United States Of America: CRC Press, Taylor and Francis Group; 2010.
5. Demyttenaere JC, Del Carmen Herrera M, De Kimpe N. Biotransformation of geraniol, nerol and citral by sporulated surface cultures of *Aspergillus niger* and *Penicillium* sp. Phytochemistry 2000;55:363-73.
6. Parshikov IA, Sutherland JB. The use of *Aspergillus niger* cultures for biotransformation of terpenoids. Process Biochem 2014;49:2086-100.
7. Chen W, Viljoen AM. Geraniol-a review of a commercially important fragrance material. S Afr J Bot 2010;76:643-51.
8. Akbar N, Saxena BK. Isolation of geraniol content from various essential oils. Asian J Exp Chem 2009;4:5890.
9. Ranitha M, Nour AH, Sulaiman ZA, Nour AH, Raj ST. A comparative study of lemongrass (*Cymbopogon citratus*) essential oil extracted by microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD) method. Int J Chem Eng Appl 2014;5:104-8.
10. Peana AT, D'Aquila PS, Panin F, Serra G, Pippia P, Moretti MD, *et al*. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine 2002;9:721-6.
11. Zengin H, Baysal AH. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. Molecules 2014;19:17773-98.
12. Perry N, Perry E. Aromatherapy in the management of psychiatric disorders. CNS Drugs 2006;20:257-80.

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