



Research Paper

Antifungal Substances from the Rhizome of *Zingiber ottensii* (Zingiberaceae)

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ABSTRACT: The rhizome of *Zingiber ottensii* has been reported to exhibit potent antifungal activities against yeast and dermatophytes. However, the active substances remain unknown. In this study, two antifungal compounds were isolated from the ethanolic rhizome extract of *Z. ottensii* through bioassay-guided separation. The isolated active substances were zerumbone (**1**) and labda-8(17),12-diene-15,16-dial (**2**) that exhibited anti-yeast activity against *Candida albicans* and anti-dermatophyte activities against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Microsporum gypseum*. In addition, the ethanolic rhizome extract of *Z. ottensii* showed fungicidal activity against *C. albicans* in the time-kill curve assay. Because of the difference between **1** and **2** in chemical classes, they might have different action modes and enhance their antifungal activities. Consequently, the rhizome of *Z. ottensii* is a promising medicinal plant for the development of antifungal preparations.

KEYWORDS: *Zingiber ottensii*, antifungal, dermatophytes, zerumbone, labda-8(17),12-diene-15,16-dial

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I. INTRODUCTION

Dermatomycoses are common fungal skin diseases caused by dermatophytes, yeasts, or mold and are predominant in the tropical and subtropical countries. Most superficial fungal infections are caused by dermatophytes and can usually be treated with topical antifungal agents. [1] Besides, it can be cured by using medicinal plants according to traditional knowledge. [2-3] In the course of our research for the investigation of antifungals from Thai medicinal plants, the rhizomes of many species in the ginger family (Zingiberaceae) exhibited antifungal activities against dermatophytes and yeast. [4] Therefore, the rhizomes of Zingiberaceae plants might be promising sources for the isolation of antifungal agents.

Zingiber ottensii Valetton is a member of the family Zingiberaceae and is widely cultivated in Southeast Asia. The plant has reddish stalk that can grow to 1.5- 2 m tall. The leaves are elliptic, narrowing at the tip. The inflorescence rises directly from the rhizomes to 0.3 m tall, and the flowers protrude from the bracts. The rhizome has purplish inside and pungent smell. This plant has been used as a traditional medicine to relieve bruises, sprains, and strains. [5] The rhizome of *Z. ottensii* has been reported to contain a number of terpenoids. [6] The objective of this study was to investigate antifungal substances from the rhizome of *Z. ottensii* that showed potent antifungal activities in the previous study. [4] The isolated active substances were tested for antifungal activities against yeast and dermatophytes using broth microdilution assay.

II. MATERIALS AND METHODS

2.1 Plant materials

The rhizomes of *Z. ottensii* were collected from Ratchaburi Province, Thailand, in November 2015. The plant was identified by the research officer of the Forest Herbarium Division, and the voucher specimen (Herbarium no. BKF 191214) was then deposited at the Forest Herbarium Division, Forest and Plant Conservation Research Office, Department of National Park, Wildlife and Plant Conservation, Bangkok, Thailand.

The antifungal activity of the ethanolic extract and the compounds isolated from the rhizome of *Z. ottensii* were tested against *C. albicans*, *T. mentagrophytes*, *T. rubrum*, and *M. gypseum*. The results of the broth microdilution assay are presented in Table 1. Zerumbone (**1**) exhibited antifungal activity with MIC values ranging from 3.13 to 50 µg/mL whilst labda-8(17),12-diene-15,16-dial (**2**) showed with MIC values ranging from 0.39 to 3.13 µg/mL.

Table 1: Antifungal activity of isolated compounds against yeast and dermatophytes

| Test Samples | Antifungal Activities (MICs, µg/mL) | | | |
|--|-------------------------------------|--------------------------|------------------|-------------------|
| | <i>C. albicans</i> | <i>T. mentagrophytes</i> | <i>T. rubrum</i> | <i>M. gypseum</i> |
| ethanolic rhizome extract of <i>Z. ottensii</i> (ZO) | 50 | 3.13 | 6.25 | 3.13 |
| zerumbone (1) | 50 | 3.13 | 12.5 | 50 |
| labda-8(17),12-diene-15,16-dial (2) | 3.13 | 0.39 | 0.39 | 0.39 |
| ketoconazole | 100 | 1.56 | 0.31 | 1.25 |

The time-kill results of the ethanolic rhizome extract of *Z. ottensii* (ZO) against the test organism *C. albicans* at test concentrations showed a reduction in the viable colony count greater than 3 log₁₀ CFU/mL whilst ketoconazole showed a slight increase in CFU/mL from the starting inoculum. (Figure 2)

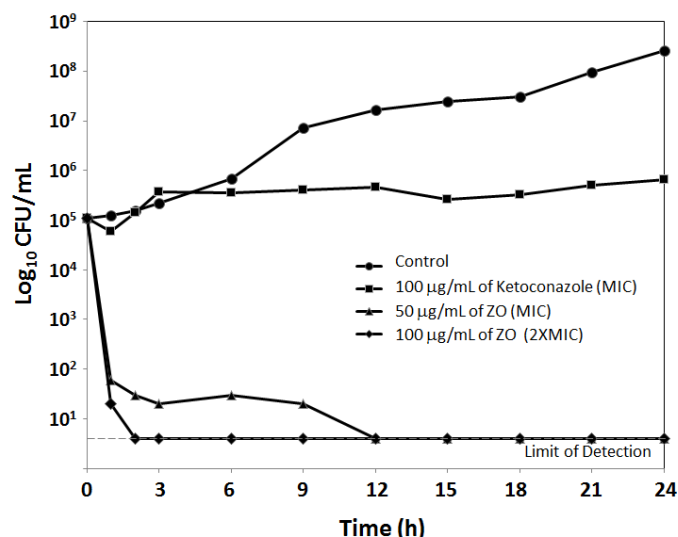


Figure 2: Time-kill curve of the ethanolic rhizome extract of *Z. ottensii* (ZO) against *C. albicans*.

IV. DISCUSSION

Many species of Zingiberaceae plants have been widely used for culinary and medicinal purposes. In traditional medicine, these plants are used to treat various kinds of diseases. For instance, *Curcuma longa* has been used for healing wounds, liver disease, jaundice, and skin disease. [13] *Zingiber cassumunar* is regularly used to treat inflammation, sprains, muscular pain, and wounds. [5, 14] In addition, *Z. ottensii* and *Z. cassumunar* are used with the same indications in Thai folk medicine. [5] Nowadays, essential oils and extracts from many Zingiberaceae species have been reported to possess antifungal and antibacterial activities.[4, 15-16] The fungal and bacterial inhibition properties of certain Zingiberaceae plants support their traditional use for treatment of wound and skin diseases.

In the present study, the ethanolic rhizome extract of *Z. ottensii* showed remarkable antifungal activity against all tested fungi and exhibited fungicidal activity against *C. albicans*, with a reduction of over 3 log₁₀ CFU/mL compared to the starting inoculum (≥99.9% initial inoculum reduction). The rhizome of *Z. ottensii* was shown to possess at least two antifungal substances, comprising zerumbone (**1**) and labda-8(17),12-diene-15,16-dial (**2**). These substances have been isolated from various Zingiberaceae plants. [11-12, 17] Zerumbone (**1**) is a monocyclic sesquiterpene ketone, first isolated from the rhizomes of *Zingiber zerumbet* [17] and also found in several Zingiberaceae species. It has been reported to possess antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and anticancer activities. [18] In a study by Jyothilakshmi et al. (2017), zerumbone isolated from the rhizome of *Z. zerumbet* has been reported to have antidermatophytic and protease-inhibiting activities

that claim for fungicidal and anti-inflammatory properties.[10] Thus, this is in good agreement with our experimental findings.

Labda-8(17),12-diene-15,16-dial (**2**) is a labdane-type diterpene, first isolated from the seeds of *Alpenia galanga*. [19] This compound can be found in other Zingiberaceae species such as *Alpenia nigra* [20], *Alpinia purpurata* [12], and *Curcuma amada* [11]. It has been reported to exhibit antimicrobial activity towards gram-negative bacteria and delay the growth of *C. albicans* by preventing substrate uptake.[20] In addition, labda-8(17),12-diene-15,16-dial (**2**) was found to possess antifungal activity against dermatophytes and showed fungicidal activity toward *C. albicans*. [11] Therefore, antifungal activity of two substances isolated in our study are in agreement with other previous studies. The result of this study could support antifungal potential of the rhizome of *Z. ottensii* for the treatment of fungal skin infection.

V. CONCLUSION

In summary, the ethanolic rhizome extract of *Z. ottensii* has broad spectrum antifungal activity against cutaneous fungi and acts against *C. albicans* in a fungicidal manner. From the rhizome extracts, two antifungal substances were isolated and identified as zerumbone (**1**) and labda-8(17),12-diene-15,16-dial (**2**), exhibiting antifungal activity with MIC values ranging from 3.13 to 50 µg/mL and 0.39 to 3.13 µg/mL, respectively. This finding indicates that the rhizome of *Z. ottensii* has potential for use to treat fungal skin infections. In addition, zerumbone (**1**) and labda-8(17),12-diene-15,16-dial (**2**) can be used as the marker substances for standardization of the rhizome extracts of *Z. ottensii*.

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