Chapter 5

Plant Secondary Metabolites for Antifusarium and Antiphytophthora

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Additional information is available at the end of the chapter

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Abstract

Plants produce secondary metabolites that are essential for survival of the producing plants such as to attract insect for pollination and defend against pest and environmental stress. Plant secondary metabolites are widely exploited by the mankind especially for medicine, one of which is to protect against infection by microorganism including fungi. Many medicinal plants have been traditionally used and/or studied for the fungicidal activity. Most of the plants studied or traditionally used as antifungi show antiphytophthora activity and some of them also active as antifusarium. Higher concentration plant extract is needed to inhibit the growth of *Fusarium* than *Phytophthora*. Considering the concentration in plant and activity as antifungi, eugenol is considered to be the most effective to be used as antiphytophthora and antifusarium. The presence of aromatic moiety, orthodioxy substitution, and double bond in the terminal of site chain is considered to be essential for the antifungal activity of the eugenol derivative.

Keywords: secondary metabolites, plant, antifungal, antifusarium, antiphytophthora

1. Introduction

Plants conduct primary metabolism to support the growth and development. In addition to primary metabolism, plant also produce and collect secondary metabolites. Compared to primary metabolites that are found in every organism, secondary metabolite has limited distribution, has been produced and collected in specific organ, and has no physiological role in the producing plants. Secondary metabolites may function in protecting the producing plant from pests and facilitate plant breeding by spreading seeds through organisms consuming

fruits produced by the plant. The mankind, however, has used secondary metabolite produced by plants since the ancient time. People use plant secondary metabolites as medicine, spices, perfumery, poison, pest control, etc.

Plants have been traditionally used as fungicide for various purposes such as food preservatives and treatment of skin diseases. Tofu that is made from soya curd is rinsed with yellow pigment of turmeric to make the color of tofu into yellow and to extend the shelf life of tofu. Garlic is traditionally used together with cassava starch to catch spore of yeast needed for fermentation of soya bean to make *tempeh* (fermented soya bean) and *tape* (fermented cassava). The addition of garlic is intended to inhibit the growth of microorganism, but the spore can still survive and can grow under suitable environment, when it is mixed with the appropriate substrates such as cassava, rice, soya bean, and other carbohydrate-containing materials to produce fermented products. *Alpinia galanga* rhizome is sliced, and the rough surface is rubbed on the skin infected by *Trichophyton*. *Cassia alata* leaves are boiled and used for bathing to treat itchy and ringworm caused by fungal infection. *Piper betel* leaves are boiled and used for washing vaginal area to treat and to prevent candidiasis. *Ageratum conyzoides* leaves is rolled up and patched on a new cut to protect the tissue from infection by microorganisms and accelerate wound healing.

Many secondary metabolites from plants have been extracted, fractionated, isolated, and studied for the antifungal activity. Volatile oil is the secondary metabolites that play many important roles in human daily life, such as perfumery, spices, essence, medicine, aromatherapy, insect repellent, and also as fungicide [1]. Many medicinal plants contain volatile oil; many of them have been traditionally used in cut healing or as natural preservatives due to their capacity to control the growth of bacteria and or fungi [2]. Coumarin is reported to be one of the antifungal compounds present in the leaves of *Ageratum conyzoides* in addition to the volatile oil components. So far not much information on the traditional use of plants as antifungi for plants infected by fungi including soil-borne pathogenic fungi such as *Fusarium* and *Phytophthora*. Plants infected by soil-borne fungi are extremely hard to eradicate. In some cases, burning of the remaining plant is the only way to eradicate the pest accompanied by replacement with different crops. The possibility of plant secondary metabolites to be developed as source for natural fungicide especially for antifusarium and antiphytophthora is explored and discussed and supported with published reports and experimental data.

2. Plants with antifungal activity

The mankind has been using plants as medicine to treat different kinds of diseases, including fungal infection. *Acorus calamus* (sweet flag), one of the medicinal plants, has antifungal activity, and the compound responsible for this activity is α-asarone that was tested on *Fusarium oxysporum* [3, 4]. *Ageratum conyzoides* that belongs to the family Asteraceae is traditionally used to treat fresh cut. It accelerates the recovery of the tissue and prevents infection. The leaves are hairy consisting ordinary trichome and glandular hair containing volatile oil. The

extract of *Ageratum* leaf contains antifungal compound that is active toward *Aspergillus niger*, *Pestalotiopsis theae*, *Rhizoctonia solani*, and *Candida albicans* [5, 6]. The responsible compounds for the antifungal activity are volatile oil components and chromene compound that was further identified as coumarin [6, 7]. Garlic (*Allium sativum*) is commonly used as spices and herbal medicine and used as antibacteria, antifungi, antivirus, antihyperlipidemia, antiplatelet aggregation, and blood fibrinolytic agent [8]. The extract of garlic is active toward *Fusarium oxysporum*, *Phytophthora capsici*, *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton rubrum*, and *Trichoderma harzianum* [9–12]. The compound that is responsible for the antifungal activity is allicin and ajoene.

Alpinia galanga is one of the medicinal plants that belongs to family Zingiberaceae and also used as seasoning. Zingiberaceae is a plant family by which the member of the family is widely used as spices and herbal medicines. The organ used is mostly the subterranean part of the plant known as rhizome. When the rhizome of *A. galanga* is sliced transversally, it produces a rough surface and traditionally used by rubbing on the skin infected by fungi, such as ringworm. The extract of the rhizome is active against *Fusarium oxysporum*, and one of the active compounds is acetoxychavicol [13]. *Curcuma domestica* known as turmeric is popular as the main spice in making curry, a popular cuisine in India and South East Asia. Turmeric extract is active toward *Phytophthora infestans*, *Exserohilum turicicum*, *Fusarium oxysporum*, and *Colletotrichum cassiicola* [14–16]. The active compounds are the component of volatile oil, i.e., eucalyptol, β-pinene, and camphor. Differed from *Curcuma domestica*, *Curcuma xanthorrhiza*, known as Java turmeric due to its bitter taste is mainly for herbal medicine. Java turmeric extract is active against *Candida albicans*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida parapsilosisi*, and *Candida tropicalis*, and the active compound is xanthorrhizol [17].

Curcuma zedoaria is also widely used as herbal medicine and even claimed as anticancer. The extract obtained from the rhizome of *C. zedoaria* has antifungal activity toward *Trichophyton rubrum*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Epidermophyton floccosum*, *Aspergillus fumigates*, *Penicillium purpurogenum*, *Trigonopsis variabilis*, *Microsporum gypseum*, *Sclerotium rolfsii*, *Geotricullar candiade*, *Fusarium oxysporum*, *Helminthosporium oryzae*, *Candida krusei*, and *Trichophyton mentagrophytes*, and the active compound is ethyl-p-hydroxycinnamate [18]. Ginger (*Zingiber officinale*) is mainly used as spices and also as herbal medicine. The rhizome contains volatile oil and pungent compounds gingerol and shogaol that are well recognized as antiemetic agent. Ginger extract is active toward *Aspergillus flavus*, *Aspergillus niger*, *Penicillium griseofulvum*, *Fusarium oxysporum*, and *Pyricularia oryzae* [5, 19] with zingerone as the active compound. Zingerone is one of the ginger oil components that belongs to the group of phenylpropanoid compounds.

Cassia alata is a shrub that belongs to the Caesalpiniaceae family. Traditionally, *C. alata* leaves are boiled and used by bathing to treat fungal infection causing skin diseases. The extract of the leaves is active toward *Trichophyton rubrum*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Aspergillus niger*, *Phytophthora notatum*, and *Fusarium solani* [3, 20–22], and the active compound was identified to be anthraquinones. Cinnamomum leaves contain volatile oil that contains cinnamaldehyde and eugenol and are reported to

be active toward *Candida albicans* [23–25]. *Cymbopogon nardus* leaves contain high quantity of volatile oil that is frequently used as insect repellent. The oil is also active as antifungal agent toward *Erysiphe cichoracearum*, *Aspergillus, Penicillium*, and *Erollium*, and the active antifungal compounds are citronellal and linalool [26]. *Eclipta alba* is commonly used as an ingredient in making hair tonic. The extract was reported to be active toward *Candida tropicalis* and *Candida albicans* [27]. *Garcinia mangostana* fruit is one of the most delicious tropical fruit. Garcinia fruit cortex that is rich in tannin and mangosteen is now commercially used as raw material for herbal medicine. Garcinia extract is active as antifungi toward *Candida albicans*, *Epidermophyton floccosum*, *Alternaria solani*, *Mucor* sp., *Rhizopus* sp., and *Cunninghamella echinulata* [28].

Piper betel leaf is traditionally chewed together with limestone and gambier by Melanesian to stain teeth and protect from infection. The leaf is also used to treat and to prevent vagina and mouth cavity from candidiasis. Betel leaf extract is reported to be active as antifungal agent for *Colletotrichum gloeosporioides*, *Botryodiplodia theobromae*, *Rhizoctonia solani*, *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. Hydroxychavicol and eugenol were reported to be the responsible antifungal compounds [29]. *Piper crocatum* is locally named as red betel; it has more bitter taste than the ordinary betel. It is considered to be more potent as herbal medicine compared to the ordinary betel; however, the volatile oil content and the antimicrobial activity were lower. The extract of red betel is active toward *Candida albicans*, *Colletotrichum gloeosporioides*, and *Botryodiplodia theobromae* [30, 31]. *Syzygium aromaticum* flower bud and leaves contain volatile oil with eugenol as the major component. The oil content of the flower bud is much higher compared to the leaves and so the eugenol content [32]. The flower bud is usually used as seasoning in cigarette-making. It is also used as local anesthesia for dental illness. The extract of clove is an active antifungi and is also active toward *Fusarium oxysporum* [4, 33, 34]. Eugenol is one of the clove oil components that has antifungal activity [35, 36].

The antifungal plants described above were extracted using methanol, and the extract obtained were tested toward *Fusarium oxysporum* and/or *Phytophthora palmivora*. The relative activities were compared, and the results are described in Section 3.

3. Plant extract antifungal activity on *Phytophthora palmivora* and *Fusarium oxysporum*

Most plants reported or traditionally used as antifungi are indeed all active toward *Phytophthora palmivora*. **Table 1** shows the antiphytophthora activity of 11 Indonesian plants that are traditionally used or experimentally reported as antifungi [36]. The capacity to inhibit the growth of *P. palmivora* can be detected by testing methanol extract that was prepared by soaking dried powder plant material at concentration 0.5%. Their activity however is different to one another. The strongest capacity is demonstrated by extract obtained from clove bud followed by *C. xanthorrhiza*, *C. zedoaria*, and *C. domestica*. These differences may be due to the concentration and the capacity of the active substances present in the individual plant

No.	Plant	Plant organ	Concentration	
			0.5%	1.0%
1	*Ageratum conyzoides* Linn.	Aerial part	+	++
2	*Cassia alata* L.	Leaves	+	+++
3	*Piper betel* L.	Leaves	++	++
4	*Allium sativum* L.	Bulb	++	++
5	*Alpinia galanga* L.	Rhizome	++	++
6	*Curcuma domestica* Val.	Rhizome	++	+++
7	*Curcuma xanthorrhiza* Roxb.	Rhizome	+++	+++
8	*Curcuma zedoaria* (Berg.) Roscoe.	Rhizome	+++	+++
9	*Zingiber officinale* Rosc.	Rhizome	++	++
10	*Cymbopogon nardus* L.	Leaves	++	++
11	*Syzygium aromaticum* L.	Flower bud	++++	++++

Note: Extract was made by maceration in methanol: +: 1–25% inhibition; ++: 26–50% inhibition; +++: 51–75% inhibition; ++++: 76–100% inhibition.

Table 1. Activity of 11 antifungal plants on ***Phytophthora palmivora***.

material. The individual active compound may be very active but present only at low concentration; consequently, the activity becomes low when the sample concentration is calculated based on the plant material. The flower bud of *Syzygium polyanthum* contains approximately 15% with eugenol content that can reach 80%. Coumarin in *Ageratum conyzoides* on the other hand only presents at very low concentration. However, for the application purpose, clove is considered potential to be used as source of antiphytophthora from plants. The rhizome of *C. xanthorrhiza*, *C. zedoaria*, *C. domestica*, and *Cassia alata* leaves can be used as alternatives. The price of the plant materials are much cheaper compared to clove bud.

Compared to *P. palmivora*, *F. oxysporum* is less susceptible toward the extract of antifungal plant. Higher concentration of plant extract is needed to observe the growth inhibition of the *F. oxysporum* culture by the extract. On *P. palmivora*, culture growth inhibition can be observed at extract concentration lower than 0.5%, whereas on culture of *F. oxysporum*, the inhibition can be observed at higher than 1%. Seven of 17 plants reported or traditionally used as antifungal agents inhibit the growth of *F. oxysporum* (**Table 2**). High inhibition activity is demonstrated by clove bud extract, and relatively high activity is shown by clove leaf extract. Inhibition by the extract of *Piper betel*, *Curcuma domestica*, *Curcuma xanthorrhiza*, *Zingiber officinale*, and *Acorus calamus* can be considered to be low [31].

Tables 1 and **2** show that clove bud and clove leaves are potential source of secondary metabolite for antifusarium and antiphytophthora. Clove bud contains 15–20% volatile oil with major components consisting eugenol (80–90%), eugenol acetate (10–15%), and caryophyllene (3%). Clove leaves also contain volatile oil, but the composition is different, and the content is much lower

No.	Plant	Plant organ	Concentration		
			2.5%	5%	10%
1	*Ageratum conyzoides* Linn.	Aerial part	—	—	—
2	*Eclipta alba*	Aerial part	—	—	—
3	*Cassia alata* L.	Leaves	—	—	—
4	*Piper betle* L.	Leaves	—	++	+++
5	*Piper crocatum*	Leaves	—	—	—
6	*Cymbopogon nardus* L.	Leaves	—	—	—
7	*Cinnamomum burmannii*	Leaves	—	—	—
8	*Syzygium aromaticum* L.	Leaves	—	+	++++
9	*Syzygium aromaticum* L.	Flower bud	+++	++++	++++
10	*Garcinia mangostana*	Fruit cortex	—	—	—
11	*Allium sativum* L.	Bulb	—	—	—
12	*Alpinia galanga* L.	Rhizome	—	—	—
13	*Curcuma domestica* Val.	Rhizome	++	++	++
14	*Curcuma xanthorrhiza* Roxb.	Rhizome	—	—	+
15	*Curcuma zedoaria* (Berg.) Roscoe.	Rhizome	—	—	—
16	*Zingiber officinale* Rosc.	Rhizome	—	—	+
17	*Acorus calamus*	Rhizome	—	++	++

Note: Extract was made by maceration in methanol: +: 1–25% inhibition; ++: 26–50% inhibition; +++: 51–75% inhibition; ++++: 76–100% inhibition.

Table 2. Activity of antifungal plant on *Fusarium oxysporum*.

compared to the clove bud. However, the oil content of clove leaf is relatively high compared to the other leaves. The oil content of clove leaves is approximately 2% with the major components which are eugenol 60% and caryophyllene 21% [37]. Clove leaves are considered to be a potential source of secondary metabolite for antifusarium and antiphytophthora. Volatile oil from clove leaves can be obtained from leaves that have already fallen on the ground; therefore, it can be collected throughout the year without disturbing the growth of the tree. In addition, the availability of clove leaves does not depend on the season and can be collected at any time.

4. The antifungal compounds from *Syzygium aromaticum*

Clove bud and leaf contain secondary metabolites that strongly inhibit the growth of *P. palmivora* and *F. oxysporum*. Under continuous extraction with hexane followed by ethyl acetate and methanol, the antifungal compounds mainly present in the hexane extract

suggesting that the active compound is nonpolar compound. Upon extraction of plant material with hexane, most volatile oil components will also present in the extract. Clove oil also demonstrates strong antifungal activity. These findings lead to the hypothesis that the antifungal compound of clove is also the component of clove oil. The major component of clove oil is eugenol. At least two compounds from the extract and the volatile oil of clove are responsible for the antiphytophthora and antifusarium activity. The two compounds were identified as eugenol and eugenol acetate. The activity of eugenol is higher than eugenol acetate.

Observation under scanning electron microscope showed that the hypha of *F. oxysporum* shrank after treated with eugenol (**Figure 1**). Higher magnification showed that the surface of hypha is no longer smooth and the cells may be leaking [38]. A number of mechanisms have been proposed to explain how eugenol acts as antifungal agent. Eugenol alters the membrane and cell wall [39] and induces leakage of protein and lipid from the cells due to the leakage of cell walls [40]. Extensive lesion of the cell membrane reduces quantity of ergosterol [41].

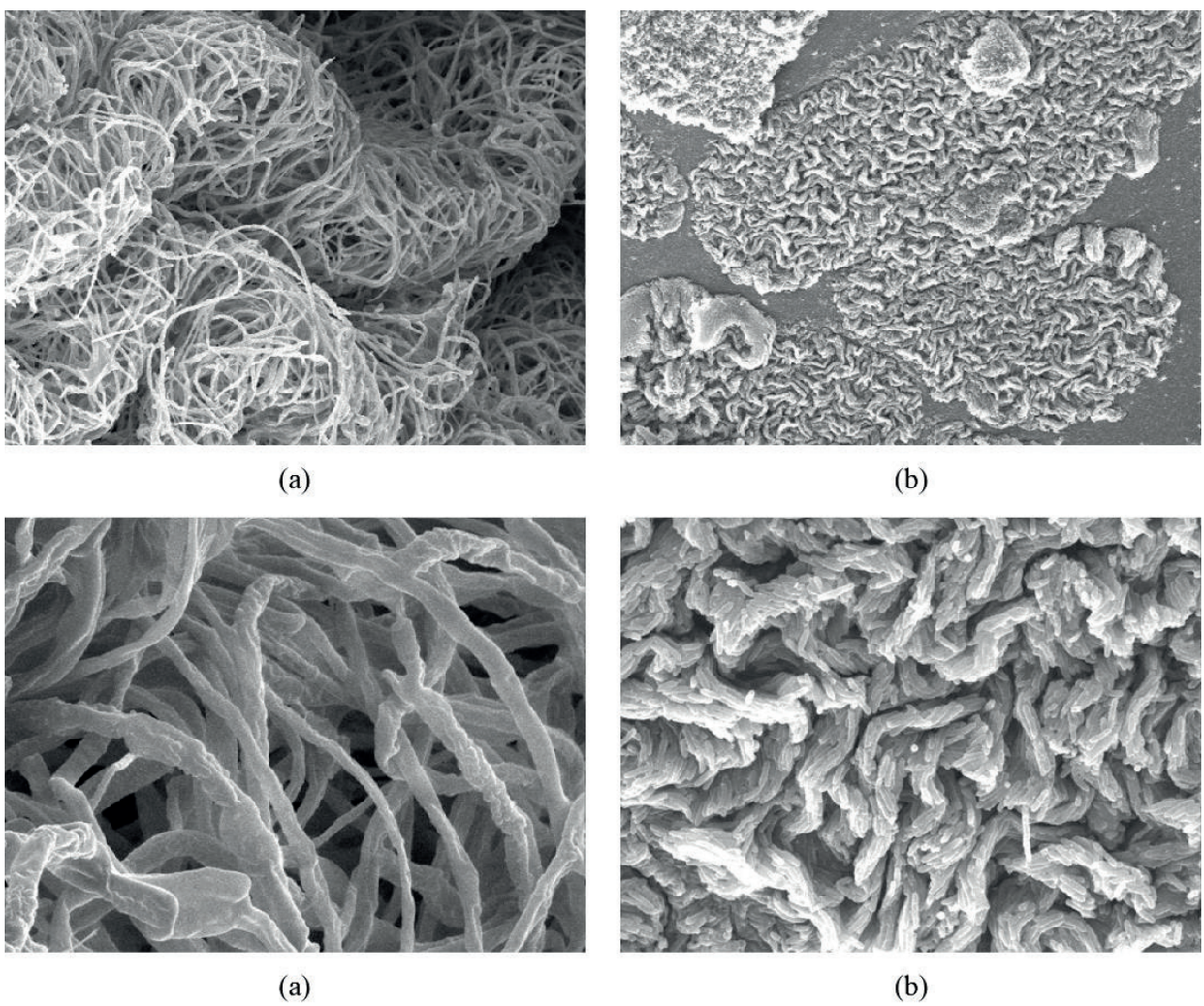

Figure 1. Scanning electron microscopy of normal *Fusarium oxysporum* (a) treated with eugenol (b) at 350× magnification (above) and at 2000× magnification (below).

It was proposed that the inhibition of ergosterol synthesis leads to the damage of cell membrane functionality and integrity [42]. However, the effect of eugenol is not reversed by osmotic support, indicating that its effect does not affect the cell wall synthesis and assembly. Furthermore, eugenol does not bind ergosterol, the main sterol of fungal membrane [43].

Eugenol is suggested to block aromatic and branched chain amino acid synthesis across the cytoplasmic membrane. Eugenol inhibits growth of yeast strain carrying a mutation in gene encoding an enzyme, a tryptophan, phenylalanine, tyrosine, and isoleucine biosynthesis pathway, in a medium supplemented with the related amino acid [44].

There are two approaches to obtain antifusarium from clove. Firstly, the secondary metabolites from clove leaves or buds can be extracted using nonpolar solvent such as hexane, petroleum ether, gasoline, or kerosene. Subsequently, the solvent is removed through evaporation leaving the concentrated extract containing antifusarium and antiphytophthora compounds. Hexane and petroleum ether have relatively low boiling point; therefore, it is easy to evaporate, and while the boiling point of gasoline and kerosene is higher than 100°C, higher temperature or lower pressure is needed to evaporate. By using extraction combined with distillation to recover the solvent, more efficient production system can be developed. Secondly, since eugenol is a component of volatile oil, the oil of clove leaves can be obtained through steam distillation by which the oil will evaporate together with steam, and upon condensation the oil will separate from water and the oil can be collected. To obtain pure eugenol, further separation processes will be needed, such as liquid–liquid extraction, vacuum fraction distillation, and chromatographic techniques.

There are some other plant metabolites having antifungal activity, and the effect is stronger than eugenol. Thymol and other components of volatile oil had been compared, and the results are as shown in **Table 3** [45]. Thymol is the component of volatile oil from *Thymus vulgaris*, herbal medicine commonly used in cough mixture. Most of these compounds demonstrate minimal inhibition concentration (MIC) above 50 ppm. The natural antifungal that demonstrates antifungal activity similar to that of commercially distributed is xanthorrhizol with MIC lower than 10 ppm [17]. Xanthorrhizol is the major component of volatile oil isolated from the rhizome of *C. xanthorrhiza*.

Compound	**Toxicity index**				
	Sclerotium rolfsii	***Rhizoctonia solani***	***Botrytis cinerea***	***Fusarium oxysporum***	***Alternaria solani***
Thymol	100	100	100	100	100
Eugenol	42.2	62.02	17.23	38.14	37.77
Methyl cinnamate	49.65	57.68	16.43	28.16	81.31
Linalool	11.8	11.33	4.45	6.03	0.85
1,8-Cineol	0.0008	0.468	—	—	—

Table 3. Relative antifungal activity of plant component compared to thymol.

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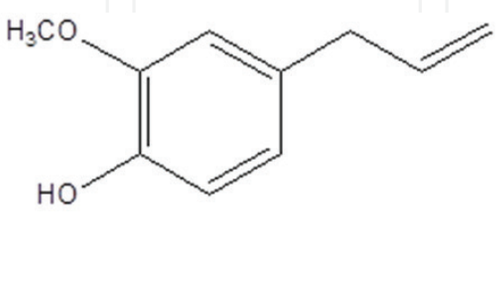
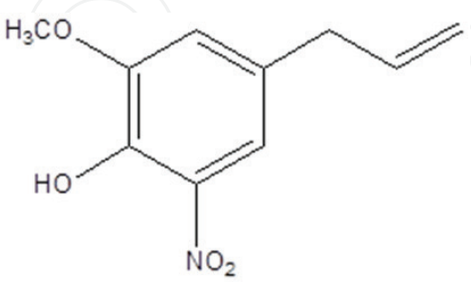
5. Structure requirement of eugenol derivatives for antifungal activity

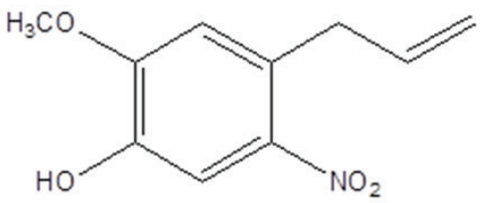
Eugenol derivatives had been synthesized and their antifungal activities evaluated [43]. Some structures and their antifungal activities are shown in **Figure 2**. It seems that the aromatic, ortho-oxygenation, and the double bond at the terminal of side chain are essential for



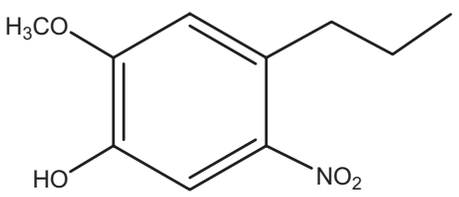
[A] MIC =125 ppm



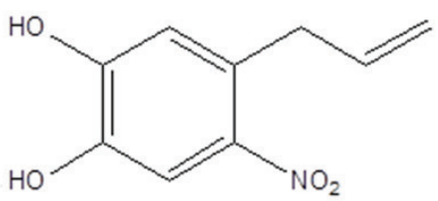
[B] MIC= 31 ppm



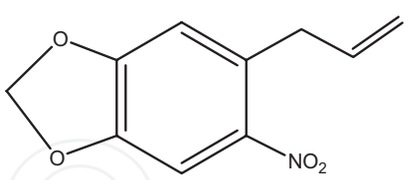
[C] MIC= 62 ppm



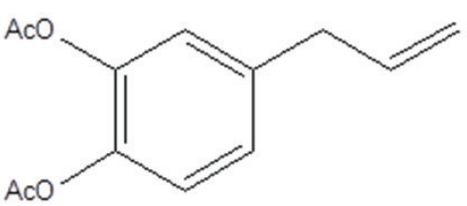
[D] MIC > 250 ppm



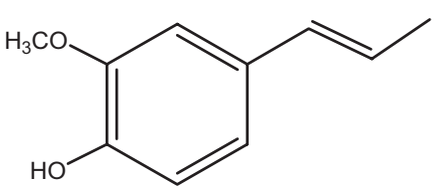
[E] MIC= 62 ppm



[F] MIC > 250 ppm



[G] MIC=125ppm



[H] MIC > 250 ppm

Figure 2. Derivatives of eugenol and their antifungal activities.

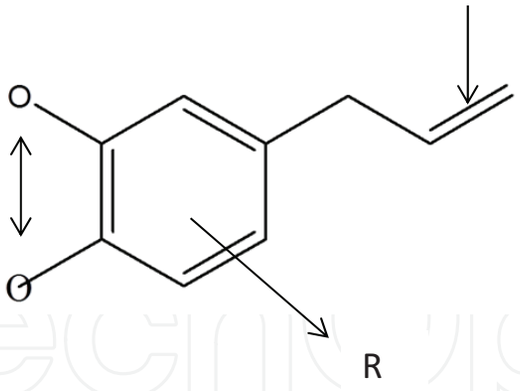


Figure 3. The important sites for antifungal activity of eugenol derivatives.

the antifungal activity. The presence of substituents on the hydroxy phenolic reduces the activity. Compound F by which the orthodioxy is connected by a methine bridge becomes inactive (MIC > 250 ppm). The absence of double bond in the side chain eliminates the antifungal activity; this is shown by compound D with MIC >250 ppm and considered to be inactive. If the position of double bond of the side chain is moved to the middle, the antifungal activity also disappears. This is demonstrated by compound H that is inactive. The presence of nitro substituent attached to the aromatic increases antifungal activity, and the nitro at ortho-position to the hydroxy group gives higher activity than at meta-position (compounds B and C).

Base on the above data, the structure requirement for eugenol derivatives to be active as a fungicide is shown in **Figure 3**.

6. Conclusion

Most extracts from plants that have been used as antifungi or reported as antifungi are also active as antiphytophthora but only few of them that are active as antifusarium. Inhibition of *Fusarium* culture growth needs higher concentration of extract compared to that of phytophthora culture. Clove bud and clove leaves are considered as potential sources for secondary metabolites for antifusarium and antiphytophthora. Clove bud and leaf contain volatile oil with eugenol as the major component. Aromatic moiety, orthodioxy, and double bound at the terminal of the side chain contribute in the antifungal activity of eugenol derivatives.

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