Review Article

INHIBITORY EFFECT OF GARLIC AND VITAMIN C ON *CANDIDA ALBICANS*

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ABSTRACT

Garlic can downregulate ECE1, a gene that regulates the production of candidalysin as a virulence factor for C. albicans. Allicin in garlic has antifungal properties because allicin can penetrate cell membranes and damage cell organelles such as vacuoles and mitochondria, causing irreversible structural and functional damage that leads to cell death. Garlic that is processed into extracts also has antifungal abilities. Purely squeezed garlic extract had a MIC of 50%, while the ethanol extract of garlic had a MIC of 40%. This difference is thought to be due to differences in the use of concentrations used and the use of extract solvents. Vitamin C can inhibit C. albicans in PBS media, there is an active metabolism and oxygen, low free iron concentration, there is mitochondrial inhibition ongoing, and in the early stationary growth phase. However, the combination of garlic petroleum ether extract and vitamin C did not show significant MIC due to the presence of glucose in the media, which could increase the growth of C. albicans.

***Keywords**: Garlic; Minimum inhibitory concentration; vitamin C; C. albicans; human and health*

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Highlights:

1. Garlic and Vitamin C effect on Candida Albicans.
2. Vitamin C has the ability to inhibit C. albicans in PBS media but it did not have significant MIC due to the presence of glucose in the media if combined with garlic petroleum extract.

INTRODUCTION

Fungi can cause disease in humans, ranging from superficial infections that only infect the skin to systemic infections that can be life-threatening, depending on the condition of the host. *Candida* is a group of fungi that generally causes 400,000 cases of systemic disease with a mortality of up to 40%. Of the many *Candida* species, *Candida albicans* (*C. albicans*) is the most common cause (Mukaremera et al. 2017). *C. albicans* is an opportunistic pathogen when the host immune system is weakened. This fungus can cause vaginitis, oral candidiasis, cutaneous candidiasis, systemic infections, and candidaemia (Santos et al. 2018).

The pathogenesis of *Candida* species is still poorly understood but infection rates are increasing rapidly (Santos et al. 2018). *C. albicans* can grow into various forms including yeast, pseudohyphae, and hyphae. The growth of *C. albicans* as a unicellular yeast is usually in a state of commensalism, whereas pseudohyphae and hyphae are usually in a state of infection (Richardson & Moyes 2015). The shape changes that occur during growth are reversible, and this plasticity is believed to be the pathogenicity of *C. albicans* (Richardson & Moyes 2015). *C. albicans* will infect change in the balance of the environment where *C. albicans* is located (Sobel 2016).

Treatment of *C. albicans* infection is usually given orally or topically. For oral infections, usually, fluconazole and itraconazole, while for topical administration clotrimazole and miconazole can be given (Sobel 2016). However, therapy with the azole group does not always work, because *C. albicans* can be resistant to the azole group. If the patient is resistant to the azole group, then alternative therapies can be used (Felix et al. 2019). Garlic can be an alternative because it has antifungal activity, not only against *C. albicans*, but also against Trichophyton, Cryptococcus, Aspergillus, Trichosporon, Rhodotorula, and Torulopsis species (Batiha et al. 2020).

By 2009, the prevalence of oral candidiasis in Indonesia had reached 84%. Oral candidiasis can occur in different age groups, with 30% to 50% of people carrying this microorganism, the number increasing with age, and about 5% to 7% experienced in infants. About 9-31% of AIDS patients have oral candidiasis and about 20% of cancer patients (Patil et al. 2015).

Garlic has an antifungal compound called Allicin, which is believed to have broad antimicrobial effects (Nakamoto et al. 2019). Garlic bulbs *(A. sativum*) contain the natural organic sulfur compound alliin. The alliinase enzyme is produced when garlic (*A. sativum*) is sliced or crushed to convert alliin to allicin (Wang et al. 2010). Allicin is an organic sulfur compound that causes its antifungal effect. Allicin is unstable and easily decomposes into allicin derivative compounds such as diallyl sulfide (DAS), diallyl sulfide (DADS), ajoene, and other organosulfur compounds. Allicin derivative compounds have antifungal properties (Bayan et al. 2014). Allicin is one of 30 sulfur compounds found in garlic. Pure allicin is insoluble in water and is an unstable compound (Marchese et al. 2016). Allicin metabolites such as diallyl disulfide (DADS) and diallyl trisulfide (DATS) have antifungal functions (Batiha et al. 2020). The combination of vitamin C with bioflavonoids has antifungal, antioxidant, and anti-inflammatory effects (Khaira et al. 2016). Vitamin C can be used as an immune booster. Vitamin C has several important roles, including being an effective antioxidant (Carr & Maggini 2017). Vitamin C combined with garlic has not been widely researched to prove an alternative therapy against *C. albicans*.

Based on this explanation, it is necessary to conduct a literature review on the inhibitory effect of garlic and vitamin C on *C. albicans*, either in combination or individually to know the inhibitory effect of garlic and vitamin C on *C. albicans*.

MATERIALS AND METHODS

All data was formulating the research, searching the extant literature used to find reference data in this literature review is internet browsing using online database in Google Scholar site, identify the review's main objective, de ine the concepts or variables or variables at the heart of their synthesis, screening a inclusion, extracting data, and analyzing data. The keywords used were garlic, garlic, minimum inhibitory concentration (MIC), vitamins C, and C. albicans. The obtained literature is then read, analyzed, and discussed. Journal limitations are those published in the last 5 years (2016-2020) and accordance with the topics raised.

RESULTS

Garlic (*Allium sativum L.*) is a plant that has been used in traditional medicine since ancient times. This plant is considered the second most common Allium species which is used as a medicine for common ailments such as fever, influenza, snakebite, and hypertension. The active ingredients of Allium species are reported to reduce the risk of diabetes and cardiovascular disease, fight infection by enhancing the immune system, and have antimicrobial and antifungal functions (Batiha et al., 2020; Goncharov et al., 2016; Marchese et al., 2016). Infections were reported most frequently.

Garlic in general has a broad spectrum of targets including antibacterial, antiviral, antifungal, and antiprotozoal. Garlic which is processed into extract has antifungal activity against *C. albicans* (Agustantina & Soekartono 2021). Garlic extract exhibits a broad fungicidal spectrum covering diverse species, such as *Candida*, *Trichopython*, *Cryptococcus*, *Aspergillus*, etc. The solvents reported having antifungal activity are aqueous, ethanol, methanol, and petroleum ether. Garlic extract acts on cell walls and causes irreversible structural damage, leading to loss of structural integrity and proliferative ability. These changes cause damage to organelles and nucleus leading to cell death. Moreover, allicin and garlic oil penetrate cell membranes, and cell organelles such as mitochondria cause organelle damage and cell death (Batiha et al. 2020).

The fungicidal action of garlic is thought to be mediated by inhibition of lipid, nucleic acid, and protein synthesis or by inhibiting the formation of *C. albicans* hyphae (Felix et al. 2019). The active ingredient responsible for being a fungicidal agent in garlic is allicin. In this regard, allicin plays a central role in the biological activity of garlic. Allicin is considered a potent compound that has a significant antifungal function which has been confirmed through in vitro assays with pure allicin, whereas so far it is in vivo activity has not been confirmed through preclinical and clinical trials (Petropoulos et al. 2018).

The garlic ethanol extract was tested by the Kirby-Bauer method with concentrations of 100%, 80%, 60%, and 40% to inhibit the growth of fungi with a diameter of 21.4 mm of the inhibition zone; 18.6 mm; 14.8 mm and 11.6 mm respectively, while the 20% concentration could not inhibit growth (Andayani & Kurniawan 2013). Garlic petroleum ether extract was inhibited at a concentration of 500 l. 750 l and 1000 l with a zone of inhibition diameter of 19.46 mm, respectively; 27.46 mm and 31.11 mm (Khaira et al. 2016). The minimal inhibition level (MIC) of the concentration of pure garlic juice that has been diluted in stages (serial dilution) is 25% by the dilution method. Meanwhile, with the UV-Vis spectrophotometric measurement method, MIC was at a concentration of 50% because at a concentration of 25% the absorbance still increased (Rambet et al. 2017). Li et al. (2016) conducted a study on the antifungal activity of garlic oil against *C. albicans*, and the results showed antifungal activity at a minimum concentration of 0.35 g/mL. In patients who received garlic therapy and were responsive, it was found that the expression of candidalysin (ECE1) was down-regulated. Meanwhile, in unresponsive patients, ECE1 showed an increase (Said et al. 2020).

Research conducted by Avci et al. (2016) yielded several findings. First, vitamin C can kill *C. albicans* in

phosphate-buffered saline (PBS) media, a medium commonly used in studies using photodynamic therapy (PDT), but there is no evidence that vitamin C kills *C. albicans* in other growth media or PBS. containing glucose. Second, vitamin C requires active metabolism and oxygen to kill *C. albicans* in PBS media. Third, the killing effect of vitamin C is increased at low free iron concentrations and in the inhibitory state of mitochondria. Fourth, the killing effect of vitamin C depends on the growth phase of *C. albicans*, the early stationary phase of *C. albicans* is sensitive to vitamin C. Garlic petroleum ether extract combined with vitamin C did not show significant MIC, only 15.4 mm (Khaira et al. 2016).

Garlic that has been processed into extract has antifungal activity against *C. albicans* (Agustantina & Soekartono 2021). The ability of garlic (A. sativum) to inhibit fungi is believed to be due to its antifungal compounds. The compound that acts as an antifungal agent is allicin. Allicin inhibits protein and nucleic acid synthesis, but lipid synthesis is completely inhibited, and inhibition of lipid synthesis may be the main reason for the antifungal activity of garlic (A. sativum) (Masoudi & Rahimi 2017). Lipids are one of the components of the Candida cell wall and are mainly in the form of phospholipids and sterols. Inhibition of lipid synthesis increases the permeability of fungal cell membranes, causing fungal cytoplasmic leakage and causing fungal cytolysis (Nurhasanah et al. 2017). The fungicidal action of garlic is thought to be mediated by inhibition of lipid, nucleic acid, and protein synthesis or by inhibiting the formation of *C. albicans* hyphae (Felix et al. 2019). Garlic oil against *C. albicans* has antifungal activity. This is indicated by the results of the study in the form of antifungal activity at a minimum concentration of 0.35 g/mL (Li et al. 2016).

The study by Said et al. (2020) showed that patients who received garlic therapy and were responsive to therapy found that ECE1 expression showed down-regulation. Whereas in unresponsive patients, ECE1 shows up-regulation activity. With the dilution method, pure garlic juice at concentrations of 100%, 50%, and 25% showed the results in the form of a clear test tube, which means the growth of *C. albicans* was inhibited. Meanwhile, by measuring the UV-Vis spectrophotometer, only the concentration of 100% and 50% decreased in absorbance, while the concentration of 25% increased, so the MIC is at a concentration of 50% (Rambet et al. 2017). Andayani & Kurniawan (2013) examined the ethanolic extract of garlic with the results of concentrations that could inhibit the growth of *C. albicans* were 100%, 80%, 60%, and 40% with an inhibitory diameter of 21.4 mm; 18.6 mm; 14.8 mm and 11.6 mm respectively. The concentration of 20% did not produce inhibition diameter. So that the MIC is at a concentration of 40%.

Vitamin C has many activities that can boost immunity. Vitamin C increases the proliferation of T and B lymphocytes. In addition, ascorbic acid is a very effective antioxidant because of its ability to donate electrons, thereby protecting important biomolecules that the body needs (proteins, carbohydrates, nucleic acids, and fats) from damage caused by oxidants (Carr & Maggini 2017). Research conducted by Avci et al. (2016) yielded several findings. First, vitamin C can kill *C. albicans* in PBS media, a medium commonly used in studies using PDT, but there is no evidence that vitamin C kills *C. albicans* in other growth media or PBS. containing glucose. Second, vitamin C requires active metabolism and oxygen to kill *C. albicans* in PBS media. Third, the killing effect of vitamin C is increased at low free iron concentrations and in the inhibitory state of mitochondria. Fourth, the killing effect of vitamin C depends on the growth phase of *C. albicans*, the initial stationary phase of *C. albicans* is sensitive to vitamin C. Another study conducted by Khaira et al. (2016) used garlic petroleum ether extract with a concentration of 1000 l, and extract of 750 l. + DMSO 250 l, extract 500 l + DMSO 500 had an inhibition zone of 31.11 mm; 27.46 mm; 19.46 mm respectively. Meanwhile, when combined with vitamin C, only extract concentration of 500 l + vitamin C 500 l had an inhibition zone diameter of 15.4 mm.

DISCUSSION

Garlic oil can penetrate the plasma membrane because it has lipophilic characteristics. Several organelles such as mitochondria and vacuoles were damaged in *C. albicans* cells and caused allicin-induced cell death. Garlic oil therapy also downregulated several proteins and genes, causing impaired cellular metabolism of *C. albicans*. This also agrees with the results of other studies which state that garlic therapy lowers ECE1 regulation. ECE1 is a gene that functions to produce a cytotoxin called candidalysin. Garlic extract using ethanol and petroleum ether as solvents showed activity to inhibit the growth of *C. albicans*. The difference in the results of the dilution and diffusion methods is thought to be because the dilution method is pure juice, while the diffusion method uses ethanol as a solvent.

Another cause could be the difference in the percentage concentration of each method. In the dilution method, from a concentration of 100% then 50% it drops to 25%, and so on. While the diffusion method, the concentration is 100%, 80%, 60%, 40% and 20%. Garlic (A. sativum) has greater inhibitory power. This could be because allicin derivatives, namely diallyl disulfide (DADS), diallyl sulfide (DAS), and ajoene

have antibacterial activity but are not as strong as allicin (Upadhyay 2016). Another study found that DADS compounds did not show good antifungal activity unless used at very high doses (Borlingus et al. 2014).

Vitamin C studied with *C. albicans* in PBS media using the PDT method showed activity to inhibit the growth of *C. albicans*. The antifungal activity was increased in PBS media, active metabolism and the presence of oxygen to kill *C. albicans* in PBS media, low free iron concentration, and inhibition of mitochondria and the early stationary growth phase. Meanwhile, the combination of garlic petroleum ether extract and vitamin C did not show significant results. This is due to research with a combination of garlic petroleum ether extract and vitamin C using sabouraud dextrose agar media which contains glucose. The presence of glucose can increase the growth of *C. Albicans*, while PBS media, it does not have glucose.

Strength and limitation

The strength of this study is that it presents a clear and concise overview of the antifungal properties of garlic and its extracts. The study provides specific information about the mechanisms by which garlic can inhibit the growth of C. albicans, including its ability to downregulate ECE1 and damage cell organelles. Additionally, the study provides quantitative data on the MIC of different garlic extracts, which adds credibility to the argument.

CONCLUSION

Garlic oil has antifungal activity. Garlic also downregulated ECE1 which is a virulence factor. The ethanol extract of garlic has a MIC at a concentration of 40%, while the pure pressed garlic has a MIC of 50%. The antifungal activity of vitamin C was increased in PBS media, active metabolism and the presence of oxygen, low free iron concentration, mitochondrial inhibition, and in the early stationary growth phase. The combination of garlic petroleum ether extract and vitamin C in SDA media did not show significant results because of the presence of glucose which increased the growth of *C. albicans*.

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Conflict of interest

None.

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Author contribution

MFL conceptualized and supervised the study, validated, reviewed, and finalized the manuscript. KA managed the administration, collected and analyzed the data, and wrote the manuscript. SAN validated, reviewed, and finalized the manuscript.

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