

Review Article

Red Ginger's Tissue Culture to Improve the Production of Phenolic Compounds Related to Antioxidant and Antibacterial Activity

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ABSTRACT

Phenolic compounds are one of the highest secondary metabolites in red ginger (*Zingiber officinale* var. *rubrum*). The most abundant and powerful phenolic compound is 6-gingerol, which is responsible for the pungent effect in red ginger. Unfortunately, the production of phenolic compounds is influenced by many factors in field-grown red ginger. Hence, it needs a more appropriate technique to produce a higher amount of phenolic compounds. One of the potential techniques is plant tissue culture, which has been known as an efficient technique to grow valuable plants *in vitro* to generate optimal production of secondary metabolites in controlled aseptic conditions. This study aims to investigate how tissue culture provides the enhancement of phenolic compounds production, related to antioxidant and antibacterial activity. It was expected to provide the defence response of plants due to the treatment added, like hormone, elicitors, manipulation of abiotic conditions, etc. In red ginger, the callus extract showed higher total phenolic content than the rhizomes. The addition of elicitors like yeast extract and salicylic acid succeeded in increasing total phenolic content in ginger. The higher total phenolic content in tissue culture-cultivated red ginger was expected to contribute to higher antioxidant and antibacterial activity. So, plant

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tissue culture is assumed to facilitate efficient production of secondary metabolites, including phenolic compounds, although its relation to antioxidant and antibacterial activity is still unclear. It may need some further research to prove a direct link between the enhancement of phenolic, antioxidant and antibacterial activity in red ginger's tissue culture.

Keywords: Antibacterial, elicitor, phenol, red ginger, tissue culture

INTRODUCTION

Red Ginger's Cultivation

Ginger (*Zingiber officinale*) is a plant widely used in Asia as a spice and herbal medicine (Suciwati & Adnyana, 2017; Widyastuti et al., 2024a; Zhang et al., 2022) for thousands of years (Miri, 2020). Based on the plant database in www.theplantlist.org (accessed on October 18th, 2024), there are eight names for the species of ginger, of which one is the accepted name and the other seven are synonyms. The accepted name is *Zingiber officinale* Roscoe, and the synonyms are *Z. officinale* var. *cholmondeleyi* F. M. Bailey; *Z. officinale* f. *macrorhizonum* (Makino) M. Hiroe; *Z. officinale* var. *macrorhizonum* Makino; *Z. officinale* f. *rubens* (Makino) M. Hiroe; *Z. officinale* var. *rubens* Makino; *Z. officinale* var. *rubrum* Theilade; and *Z. officinale* var. *sichuanense* (Z. Y. Zhu, S. L. Zhang & S. X. Chen).

Commonly, gingers are categorised into three varieties based on the differences in size and colour of their rhizomes. There are white giant ginger (*Z. officinale* Rosc. var. *officinale*), small white ginger (*Z. officinale* Rosc. var. *amarum*), and red ginger (*Z. officinale* Rosc. var. *rubrum*) (Zhang et al., 2022). All three varieties are reproductively sterile. They are only propagated via their rhizomes, vegetatively. However, the vegetative cultivated red ginger is susceptible to *Ralstonia solanacearum*, *Pythium* spp., *Fusarium* spp., and some other soil microbes, which cause the rhizome rot (Miri, 2020). Red ginger, in some places known as "sunti" ginger. Some synonyms of red ginger's scientific names are *Z. officinale* Roscoe var. *sunti* Val., *Z. amomum* L., *Z. cholmondeleyi* (F. M. Bailey) K. Schum, *Z. missionis* Wall., and *Z. sichuanense* (Nurdyansyah & Widyastuti, 2022).

Red ginger has red-coloured rhizomes, different from the two other varieties, which have white-coloured rhizomes. The red colour is related to its anthocyanin content (Widyastuti et al., 2024a). Red ginger is included in the division of Spermatophyta, subdivision of Angiospermae, class of Monocotyledoneae, order of Zingiberales, and Zingiberaceae family. It is mainly cultivated in Asia (Suciwati & Adnyana, 2017), especially in China, Indonesia, and Malaysia (Zhang et al., 2022). The conventional cultivation of red ginger uses its rhizome to grow a new plant. However, it possesses some weaknesses such as low proliferation rate, long growth time, and soil pathogen infection resulting in damage to the damage in rhizome. Red ginger cultivation has to face the limitation of superior seeds, so it may need other techniques to cultivate red ginger (Nurdyansyah & Widyastuti, 2022).

The selection of rhizomes to use as seeds for conventional cultivation has to be done. Big fresh rhizomes with no wrinkles and no scars are usually used as seeds to cultivate red ginger vegetatively. Red ginger's cultivation mostly is in the end of the rainy season to avoid decomposition due to high rain intensity. To maintain the growth of red ginger, the plant has to be watered, fertilised, and weeded. Red ginger can be harvested after 3 to 4 months of planting (Nurdyansyah & Widyastuti, 2022).

Red Ginger's Bioactive Compounds

Red ginger contains many bioactive compounds with different biological effects. Its essential oils and oleoresins produce a strong, pungent, and sour flavour. Fresh rhizomes possess lipids (0.9%), minerals (1.2%), proteins (2.3%), fibre (2.4%), carbohydrates (12.3%), and water (80.9%) (Beristain-Bauza et al., 2019). It has antioxidant agents which correlated to its phenolic and flavonoid contents. Some phenolic compounds known in red ginger are 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol, 10-shogaol (Figure 1), paradol, and zingeron. On the other hand, it is already well-reported that all varieties of ginger also contain a large amount of flavonoids, which are natural antioxidants and can reduce the risk of heart disease, cancer, and high blood pressure (Jiang et al., 2017). Some flavonoid compounds known are rutin, quercetin, catechin, and epicatechin (Widyastuti et al., 2024a).

Red ginger possesses about 169 bioactive compounds, including monoterpenes, sesquiterpenes, diterpenes, flavonoids, phenolic compounds, vanilloids, etc. Moreover, it also contains vitamins, amino acids, and some trace elements such as copper, iron, zinc, chromium, manganese, nickel, etc (Zhang et al., 2022). Total phenolic and flavonoid content in red ginger is known to be higher than that of white ginger (Ghasemzadeh et al., 2010). Hundreds of its bioactive compounds were classified into three categories: gingerols, volatile oils, and diarylheptanoids. Gingerols belong to the phenols that include gingerone, shogaols, and paradols. Whereas, the volatile oils include monoterpenoids and sesquiterpenoids. The diarylheptanoids are a class of derivatives which have a 1,7-diarylheptane skeleton (Jiang et al., 2017).

The 6-gingerol is the most abundant and plays a role as the most powerful pharmacologically active phenolic compound found in the rhizomes of red ginger (Widyastuti et al., 2025). While the other gingerols, like 4-, 8-, 10, and 12-gingerols, are found in lower concentrations (Beristain-Bauza et al., 2019). This 6-gingerol possesses some biological activities such as anti-inflammatory, antioxidant, anti-mutagenic, anti-carcinogenic, etc. 6-gingerol is also known to have a therapeutic effect in osteoarthritis by attenuating the oxidative stress as well as downregulating the pro-inflammatory mediators. It also inhibits the metastasis and induces cell death in cancer cell lines (Alsahli et al., 2021).

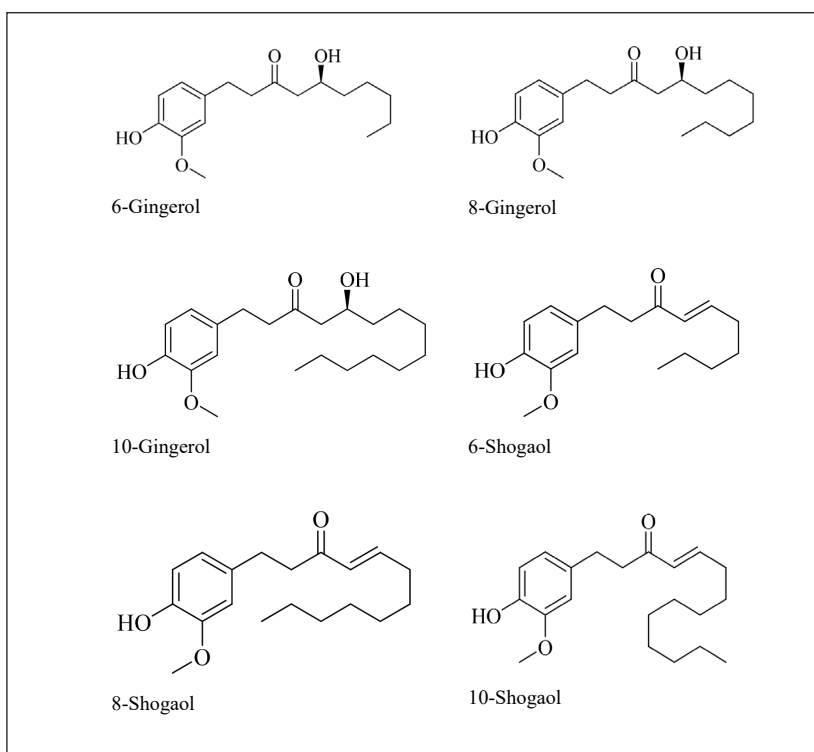


Figure 1. The main phenolic compounds in red ginger (Zhang et al., 2022)

Gingerols, including 4-, 6-, 8-, 10-, and 12-gingerols, are dominant in fresh rhizomes of red ginger. However, the 6-, 8-, and 10-shogaols are dominant in dry rhizomes (Hossain et al., 2019; Zhang et al., 2022). Gingerols and their derivatives play an important role as pungent bioactive compounds (Beristain-Bauza et al., 2019; Yudthavorasit et al., 2014). Based on GC-MS results, red ginger oils contain three monoterpenes (14.5% champene, 14.3% geranial, and 13.7% geranyl acetate) and 47 sesquiterpenes.

PLANT TISSUE CULTURE FOR RED GINGER'S CULTIVATION

Benefits of Plant Tissue Culture

Plant tissue culture is a technique to optimise plant production by micropropagation, genetic transformation, gene modification, germplasm preservation, pathogen eradication, etc. This technique is an important tool in agriculture, industry, and plant breeding, etc (Custódio et al., 2023). Plant tissue and organ culture can be described as the manipulation of cells and/or organs in aseptic conditions, grown in a suitable culture medium, and under controlled conditions like temperature, humidity, light, etc (Dias et al., 2016). It uses culture media

with some nutrients and hormones, controlled aseptic conditions, and optimal abiotic conditions for the growth of cells, tissues, and organs.

Plant tissue and organ culture can be initiated from any part of the plant based on its totipotency. For example, the shoot tip is known as an ideal explant for micropropagation or direct shoot regeneration (Bhoite & Palshikar, 2014). Plant tissue culture supports rapid multiplication of rare plant genotypes, plant genome transformation or modification, and production of disease-free plants in a shorter time (Mehaboob et al., 2019), and production of important plant-derived secondary metabolites (Espinosa-Leal et al., 2018). Furthermore, the addition of plant growth hormones (synthetic or natural phytohormones) can manipulate the plant development (Phillips & Garda, 2019). The rare plant species which economically important can be produced in larger quantities through tissue culture (Bhoite & Palshikar, 2014).

Plant tissue culture is also an efficient alternative technique to manipulate the production of secondary metabolites due to the response to different types of stress given in the culture (Winson et al., 2021). Plant secondary metabolites are mainly used across industries, such as cosmetics, pharmaceuticals, fragrances, supplements, dyes, or flavours. The biochemical properties extracted from plant tissue cultures are also easier to purify, so they can reduce the cost of production (Babu et al., 2020; Humbal & Pathak, 2023). So, the plant tissue culture is one of the promising techniques to meet the growing demands of plant secondary metabolites (Ramirez-Estrada et al., 2016). This technique allows better control of the plant development, production of some specific tissues, and also the activation of biochemical pathway which trigger the synthesis of specific secondary metabolites (Cardoso et al., 2019).

Plant Tissue Culture to Enhance Red Ginger's Bioactive Compounds

The secondary metabolites production of plants usually comes in a low concentration (< 1% dry weight) due to environmental factors (Winson et al., 2021). Plant tissue culture emerges as a viable biotechnological technique for the production of important secondary metabolites of plants, which can be used in the pharmaceutical or biological function (Espinosa-Leal et al., 2018). Cultivation of plant cells through in vitro tissue culture could be potentially competitive for the effective production of secondary metabolites. The number of phytohormones added plays a vital role in the enhancement of callus growth, leading to the enhancement of secondary metabolites production (Kumari et al., 2016).

The accumulation of plant secondary metabolites is a defence mechanism against unstable conditions, like abiotic stresses, pathogen attacks, bacterial or fungal infections, or others (Mathur, 2018). With plant tissue culture, the production of plant secondary metabolites can be improved by the treatment of elicitors (Ali et al., 2018), both biotic or abiotics elicitor, which can activate the signal compounds of plant defence responses (Mathur, 2018). The addition of elicitors can overcome the limitations of plant tissue culture,

which commonly obstruct the plant's commercial values (Narayani & Srivastava, 2017). Elicitors can also be added to cell suspension cultures so that the culture will face physical or chemical stresses. Those stresses trigger the production of secondary metabolites, which may not be formed in naturally grown plants (Fazili et al., 2022).

Elicitation mimics natural conditions when plants respond to pathogens or other abiotic disturbances by producing defensive secondary metabolites. It has been proven as a successful technique in improving the production of secondary metabolites over whole plants (Winson et al., 2021). Hence, plant tissue culture means to facilitate the production of interesting secondary metabolites in vitro (Nabi et al., 2021). The biotic and abiotic elicitors have been used in plant tissue culture to induce and improve the different production of secondary metabolites by reducing the cultivation time required to enhance their productivity (Elshahawy et al., 2022). Elicitor-induced signal transduction pathways, which result in the production of secondary metabolites (Figure 2) (Narayani & Srivastava, 2017).

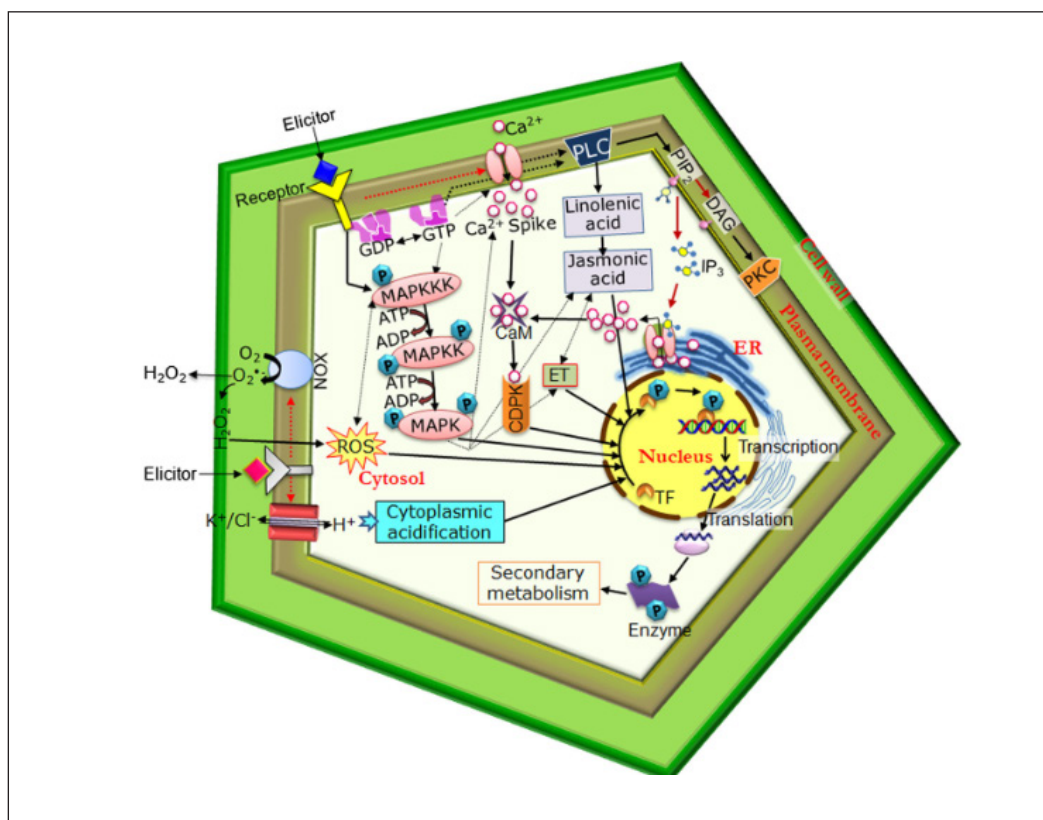


Figure 2. The schematic illustration of the induction of signal transduction pathway in the production of plant secondary metabolites through the addition of an elicitor (Narayani & Srivastava, 2017)

Elicitation is a strategy in plant tissue culture to enhance the production of bioactive compounds through activating the plant defence mechanism for scavenging the ROS (reactive oxygen species). The activation of genes to produce various enzymes that are involved in plant defence mechanisms will modulate the production of secondary metabolites related to those defence responses (Bakhtiari & Golkar, 2022). The way of elicitor application (Złotek et al., 2019), duration, concentration, and nature of elicitor (Figure 3) are some important influencing factors to induce the optimal production of secondary metabolites (Al-Khayri & Naik, 2020). The elicitation in ginger's cultures was proven to show a significant escalation of antioxidant activity, compared to the unelicited culture (Ali et al., 2018). Elicitation can encounter the limitations of low secondary metabolite production in plant tissue culture. It generates higher amounts of specialised compounds compared to others with no elicitors. The use of biotic or abiotic elicitors stimulates molecular responses in plant tissue cultures, which lead to the enhancement of secondary metabolites production (Arya et al., 2022).

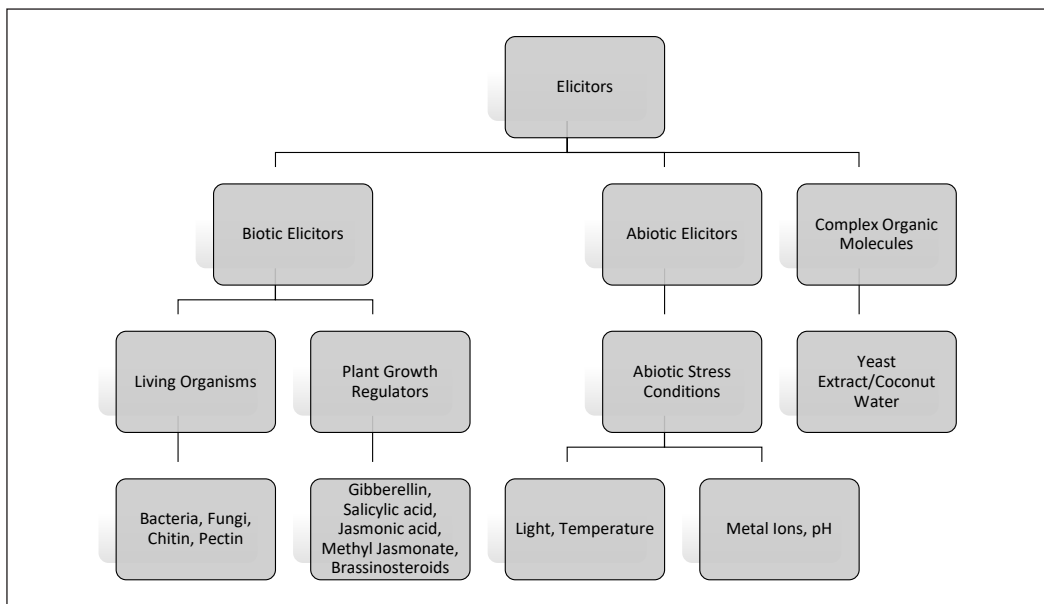


Figure 3. A graphical classification of classes of elicitors (Nabi et al., 2021)

PRODUCTION OF PHENOLIC COMPOUNDS IN RED GINGER

Enhancement of Phenolic Compounds with Plant Tissue Culture

All varieties of ginger are known as plants with high phenolic compounds (Widayat et al., 2018). The total phenolic compounds in red ginger have a positive correlation to its antioxidant activities. The high level of total phenolic and flavonoid content indicated high

antioxidant activities in Halia Bara ginger (Ghafoor et al., 2020; Johari & Khong, 2019). Unfortunately, the production of phenolic compounds is decreased with elevation (Nataraj et al., 2022). So, in red ginger, it is quite challenging to take advantage of its phenolic compounds like gingerol and shogaol from field-grown rhizomes. It requires a large amount of red ginger's rhizomes to meet the needs of the pharmaceutical and industrial sectors, which leads to bigger production costs.

The phenolics are usually found in limited amounts in naturally grown plants, and their biosynthesis is sensitive to stress conditions, both biotic and abiotic stresses, like pathogen-induced damage, extreme temperatures, nutritional deficiencies, drought, etc. Hence, plant tissue culture offers a reliable system to provide phenolic biosynthesis under controlled conditions (García-Pérez et al., 2020).

Plant tissue culture has been known as an effective technique to improve the growth and metabolism rates. The culture media support cell growth initiation to faster cell proliferation, so that the secondary metabolites production will also increase. Red ginger with a low proliferation rate can utilise this technique to optimisation of secondary metabolites production (Widyastuti et al., 2024a). The use of plant tissue culture on Murashige and Skoog (MS) media with a high concentration of sucrose (about 60-90 gL⁻¹) promotes the differentiation of microrhizomes in ginger culture. Furthermore, optimised levels of hormones like NAA and 2,4-D promote the calli production of ginger culture (Rani et al., 2022). The plant tissue culture is also expected to produce secondary metabolites at lower costs (Mamdouh & Smetanska, 2022).

To increase the production of phenolic compounds in red gingers, the rhizome's buds can be used as an explant to induce callus. The callus culture can lead to increasing of phenolic compounds production. Furthermore, cell suspension cultures also maintain cells in suspensions, which enhances the synthesis of phenolics by providing optimal growth conditions. Applying biotics or abiotics elicitors is also known to stimulate the higher production of phenolic compounds in red ginger's tissue cultures. *Aspergillus niger* and *Fusarium oxysporum* were known to be used as biotic elicitors in *Echinacea purpurea* callus and succeeded in enhancing its production of phenolics, flavonoids, and antioxidant capacity (Elshahawy et al., 2022). The use of 0.1% yeast extract and 10 µM jasmonic acids was proven to increase the total phenolic content in lovage leaves (*Levisticum officinale* Koch) cultures (Złotek et al., 2019).

In white ginger tissue culture, the treatment of callus with elicitors like yeast extract, salicylic acid, and glycine was proven to significantly enhance the total phenolic content (Ali et al., 2018). The development of red ginger's callus in MS media supplemented with 2,4-D and Kinetin generates higher total phenolic content than rhizomes (Widyastuti et al., 2024b). Cell suspension culture of Sabah snake grass (*Clinacanthus nutans*) generates higher total phenolic content than leaf and steam extracts (Bong et al., 2021). The callus

induction of *Nepeta binaloudensis* in ½ MS media supplemented with the combination of BAP and NAA has been known to produce optimal concentrations of secondary metabolites like phenolic, flavonoid, and tannin (Sagharyan et al., 2020). *Lepidium sativum* callus elicited with 250 mg/L chitosan performs higher accumulations of total phenolics, phenylalanine (PAL) activity, reactive scavenging ability (RSA), and relative callus fresh weight (Bakhtiari & Golkar, 2022).

Phenolic Compounds Related to Antioxidant and Antibacterial Activity

The phenolic compounds are molecules which possess at least one phenol unit. It can be obtained from marine organisms, bacteria, fungi, but mostly from plants. Phenolic compounds are classified into some subcategories based on their chemical structure, including simple phenols, phenolic acids, flavonoids, tannins, coumarins, lignans, curcuminoids, quinones, and stilbenes (Ecevit et al., 2022). Polyphenols have aromatic carboxylic acids with multiple phenolic hydroxyl groups, which are substituted on one ring of benzene with concomitant features (Fu et al., 2020). Polyphenols reflect the most general phytochemicals with various beneficial health effects. Phenolics have some biological activities, from antioxidants to antiproliferative compounds (Lobiuc et al., 2023).

The phenolic acids derived from plants have been reported to have antioxidant, antimicrobial, anti-inflammatory, and pro-coagulant properties. It provided some antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* at appropriate concentrations that were possible for integration into polymeric biomaterials (Liu et al., 2020). Phenolic compounds derived from plants have demonstrated antibacterial properties against some foodborne pathogens, like *Salmonella* sp. (Widyastuti et al., 2026), *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, and *E. coli*. Phenols synergise with antimicrobials in clinical matters to increase the antimicrobial effectiveness and mitigate resistance in foodborne pathogens (Padilha da Silva et al., 2024). A type of phenol, gallic acid extracted from *Hibiscus sabdariffa*, needs 50 mg/L to kill *Salmonella enterica* serovar Typhimurium (SeleneMárquez-Rodríguez et al., 2020).

The antioxidant potential contributes to the antibacterial activity as well as free radical scavenging activity (Pradhan et al., 2021). Phenolic acids have antimicrobial properties correlated to their hydrogen-donating groups, which could be reactive to oxidants, then form the resonance-stabilised phenoxyl radicals. Those will provide antioxidant activity to improve healing outcomes. Phenolic acids are shown as multifunctional molecules to improve the healing outcomes and reduce drug-resistant infections. However, they were commonly studied in relation to foodborne illness and plant pathogens. The study about antimicrobial properties of phenolic acids in clinical bacterial infections and wound bacteria is just limited (Liu et al., 2020).

Red ginger with its anthocyanin has the structure of flavylium cation (AH⁺), which correlates to its antioxidant activity (Widyastuti et al., 2024a). Anthocyanins are one of the phenolic compounds that are characterised as the largest group of water-soluble pigments. They have various biological activities, including their ability to deactivate reactive oxygen species (ROS) as one of the free radicals, improve the superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities as antioxidant enzymes, increase glutathione content, and reduce the DNA oxidative damage (Abdurrahim et al., 2023). Those activities mean that the presence of anthocyanins in red ginger can increase its antioxidant and antibacterial activities. The combination of gingerols and anthocyanins in red ginger was estimated to trigger a synergistic antioxidant effect against oxidative stress (Abdurrahim et al., 2023).

The phenolic acids have antibacterial activity against Gram-positive and Gram-negative bacteria. The Gram-positive bacteria are most sensitive to phenolic compounds due to their peptidoglycans, which leads to the lack of an outer membrane. However, their antibacterial activity depends on the bacterial strains, species (Lobiuc et al., 2023), and the chemical structure of compounds, like chain length, number of benzene rings, and position of substituents on the core of benzene rings. The phenolic acids show bactericidal actions, resulting in bacterial cell death (Ecevit et al., 2022). The undissociated forms of phenolic acids cross through cell membranes with passive diffusion, then acidifying the cytoplasm and causing the outflow of the essential intracellular constituents of the cell, including nucleic acids, proteins, and inorganic ions like phosphate or potassium (Lobiuc et al., 2023), resulting in bacterial cell death (Ecevit et al., 2022). The phenolics have several mechanisms to kill the bacterial cells (Figure 4), including blocking the enzymatic activity

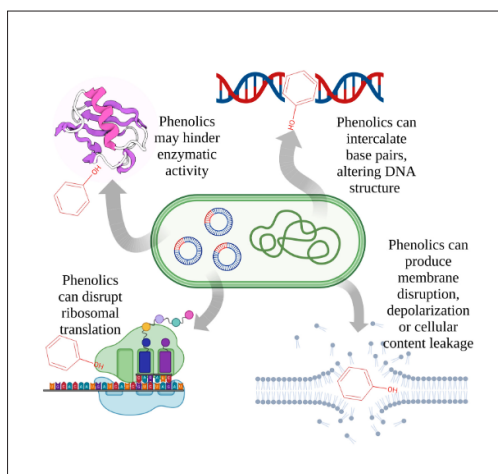


Figure 4. The mechanisms of antibacterial activity of phenolic compounds (Lobiuc et al., 2023)

of the bacterial cells, inserting base pairs or altering the DNA structure, interrupting the ribosomal translation, and producing cell membrane disruption, depolarisation, or cellular leakage (Lobiuc et al., 2023).

The enhancement of phenolic compounds production of red ginger through plant tissue culture provides the improvement of its antibacterial activity. The phenolic compounds in blackcurrant have been proven to have antibacterial activity against *E. coli*, *S. aureus*, and *Salmonella* sp. Furthermore, the anthocyanin in blackcurrant, just like in red ginger, showed moderate antibacterial

activity against *E. coli* and *S. aureus* (Zhao et al., 2021). With the higher total phenolic content (Widyastuti et al., 2024b) and antioxidant activities in red ginger callus through *in vitro* cultivation, it might also be predicted to have antibacterial activity against several bacterial strains.

CONCLUSION

Plant tissue culture is known to be an alternative cultivation for vegetatively weak red ginger. This technique allows for increased production of secondary metabolites with other characteristics compared to fieldgrown red gingers. The enhancement of secondary metabolite production can be affected by some factors, such as biotic and abiotic conditions, addition of hormones, treatment of elicitors, etc. The red ginger's callus extract has been known to have higher total phenolic content than the field-grown rhizome's extract. The elicitors like yeast extract and salicylic acid are known to generate higher total phenolic content and antioxidant activity of red ginger's callus, which may lead to higher antibacterial activity. Furthermore, the relation among phenolic, antioxidant, and antibacterial activity may be synergistically interconnected, but there are possibilities that the antioxidant and antibacterial activity are not only related to total phenolic content but also to other internal or external factors of the cultures.

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