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REVIEW ARTICLE
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Potential for Production of Kencur (*Kaempferia galanga* L.) Secondary Metabolites with Auxin and Cytokinin *in vitro*

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ABSTRACT

In Indonesia, galangal is a common rhizome plant that is grown in yards. Galangal can be used as a spice, a base for traditional Indonesian herbal medicine, or as an ingredient in other pharmaceutical products. In reality, the production of Kencur plants should be focused on the quality of the plant's rhizomes, which will subsequently serve as raw materials for the pharmaceutical industry, rather than just quantity, as is typically the case. There are a lot of beneficial secondary metabolites in the galangal rhizome. Kencur produces several significant bioactive ingredients, including ethyl P-Methoxycinnamate (EPMC). Issues in the field revealed several barriers to galangal cultivation, such as the long harvesting season, the lengthy growing season for planting material, and low quality. *In vitro* cultivation is one method that can be used to create Kencur's secondary metabolites. The key to successful *in vitro* cultivation is the administration of growth regulators. More research is needed to determine the best growth regulator type, combination, and concentration for high-quality *in vitro* Kencur production. The primary objective of this review is to explore the potential for producing secondary metabolites from Kencur using *in vitro* techniques with the application of auxin and cytokinin.

Keywords: Auxin, Ethyl P-Methoxycinnamate (EPMC), *Kaempferia galanga* L., Cytokinin

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INTRODUCTION

Kencur or galangal (*Kaempferia galanga* L.) is a plant belonging to the ginger root family (Zingiberaceae) and has high economic value (Dalilah et al., 2023). Farmers are being encouraged to attempt to provide galangal in large quantities as a result of the improving market conditions for galangal plants, particularly from medicine factories. In Indonesia, galangal is used in a variety of contexts, including homes, traders, traditional herbal medicine companies, and the pharmaceutical sector. Galangal is widely used as a spice and in traditional medicine because of its unique flavor and aroma. Galangal rhizomes are widely used as spices, aromatherapy, and cosmetics, and they can be a component of at least 59 different types, according to Preetha et al. (2008).

Ginger (50.36%) is the most frequently utilized medicinal plant in Indonesia, with galangal (48.77%), temulawak (39.65%), meniran (13.93%), and pace (11.17%). In addition to the aforementioned medicinal plants, up to 72.51% of the population utilizes the other types of medicinal plants. In both rural and urban areas, 95.60% of Indonesians who use herbal medicine report benefits across all age groups and socioeconomic statuses. Indonesia is expected to become a pharmaceutical industry in the field of natural ingredients, one of which is the production of Kencur, according to Ministery of Health Regulation (PMK) No. 17 of 2017 concerning the Action Plan for the Development of the Pharmaceutical and Medical Devices Industry (Ministry of Health Regulation, 2017). According to BPS data (2022), Indonesia's production of Kencur plants increased dramatically from 35,296 tonnes in 2019 to 54,408 tonnes in 2021. The Covid-19 pandemic is one of the reasons behind the rise in Kencur production; it has raised public awareness and raised the cost of Kencur, encouraging many farmers to grow Kencur plants.

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Due to its many applications, the galangal plant has undergone extensive cultivation development with modifications made to achieve the intended end product. The primary goals of Kencur cultivation—which produces galangal rhizomes used as a source of medicinal plants—are production, quality, and active ingredient content. Plant material with guaranteed quality and production level is necessary for the acquisition of high-quality galangal rhizomes. The largest chemical compounds, including carvon, pentadecan, eucalyptol, metal cinnamate, and ethyl P-methoxycinnamate (EPMC), can be observed in galangal rhizomes (Umar et al., 2012; Adianingsih et al., 2023). Because EPMC compounds can shield the skin from sunlight and be used as raw materials for perfume, they are extensively utilized as antibacterials and ingredients in the cosmetics industry. Aside from that, galangal rhizomes' compounds are widely employed in traditional medicine to treat respiratory and digestive ailments as well as raw materials for cosmetics (Rosita et al., 2007).

The presence and growth of plants in the field, which are influenced by a variety of environmental condition factors, has a significant impact on the production of secondary metabolites in galangal rhizomes for the needs of pharmaceutical industry factories (Shofiyani and Purnawanto, 2010). The cultivation of galangal in Indonesia is still limited to home gardens and lacks quality control, so more innovation and development in this area is required. The use of tissue culture technologies, such as callus culture, is one attempt to generate secondary metabolites in large quantities. Preetha et al. (2013) states that secondary metabolite production can be accomplished through *in vitro* culture, in addition to plant conservation and propagation. Through this technique, the production of secondary metabolites does not depend on plant sources in the field.

Conventional galangal cultivation takes a considerable amount of time—at least six to twelve months. Chithra et al. (2005) state that for a cultivation area of approximately 1 ha, 800-1600kg of galangal planting material is required to achieve optimal production. In Indonesia, where food crops occupy a large portion of the agricultural land, *in vitro* propagation of galangal is highly common due to certain conditions. *In vitro* cultivation has advanced significantly in India to produce medicinal plants that are used as raw materials for the pharmaceutical industry (Shirin et al., 2000).

In vitro cultivation relies on growth regulators among other factors. To cause cell division, various growth regulators is required, including those belonging to the auxin, cytokinin, and gibberellin groups (Preetha et al., 2016). Growth regulators of the appropriate kind and concentration can be used to promote the formation of calluses.

The development of quick and efficient cultivation is essential considering the current demand (for both medicinal and economic value) and the challenges associated with plant propagation. Pharmacology could benefit from *in vitro* techniques to circumvent the issues related to traditional field maintenance.

Objective of this review is to explore the potential for producing secondary metabolites from *Kaempferia galanga* L. using *in vitro* techniques with the application of auxin and cytokinin. The following sections aim to discuss the importance of Kencur, analyze the active compounds in Kencur rhizomes, examine the challenges in Kencur cultivation, investigate *in vitro* techniques, and evaluate the effects of auxin and cytokinin.

Potential of Kencur in Indonesia

In Indonesia, galangal cultivation has a long history and is still widely practiced in a number of areas. An essential component of many traditional Indonesian dishes and herbal products like herbal medicine is galangal. In addition, galangal has bioactive substances that are useful in the cosmetic and pharmaceutical sectors. In Indonesia, galangal is traditionally grown by slicing and cleaning the seeds before planting them in prepared soil (Adianingsih et al., 2021). Galangal can be harvested after nine to ten months, at which point the rhizomes can be exported or sold in nearby markets. Indonesia is currently seeing innovation and development in the cultivation of Kencur. Numerous research projects have been undertaken to produce Kencur in more productive and efficient methods. For instance, Kusnadi et al. (2021) applied organic fertilizer cultivation technology; Nurcahyani and Hidayati (2019) deployed planting media technology in hydroponic cultivation to utilize narrow land; Saitama et al. (2024) applied Kencur and teak agroforestry; Zaini et al. (2021), focus on K_2O fertilization and shade; while Kurniawan et al. (2021) MgSO4 fertilization and shade.

Indonesia still has a number of issues with the development of medicinal plants. These are some of the issues impeding Indonesia's efforts to cultivate medicinal plants. As stated by Rohman (2018), Yunus (2019) and Soekarjo (2017):

1. Government neglect and lack of support: Government spending on the research and development of medicinal plants has stayed comparatively low in recent years. This has an impact on Indonesia's inability to increase the quantity and quality of its medicinal plant production. The quality of medicinal plants is one aspect of biopharmaceuticals.
2. Concentrate solely on output: Few Indonesian medicinal plant farms emphasize quality; instead, they aim to maximize output volume.
3. Lack of knowledge and awareness among the general public: The market for medicinal plants is small and their use is less common due to a lack of knowledge about their advantages.
4. Variations in climate and environmental factors: The development and harvesting of medicinal plants in Indonesia is impacted by variations in climate and environmental factors. Certain medicinal plants may grow more slowly or not at all in harsh environments.
5. Overuse of chemicals: Excessive use of pesticides and herbicides can harm the quantity and quality of medicinal plants as well as have negative effects on human health. The absence of dangerous chemical residues is a crucial feature of medicinal plants.

Rhizome Content of Kencur

Medicinal plants have long been employed in Southeast Asian and Indonesian traditional medicine. Sesquiterpenes, flavonoids, phenolic acids, and essential oils are a few of the active ingredients in Kencur. Galactol has anti-inflammatory, antioxidant, antimicrobial, and anticancer properties (Adianingsih et al., 2023). Additionally beneficial to digestion, galangal can ease stomach aches, prevent nausea and vomiting, and lessen constipation. Furthermore, a number of studies have demonstrated the benefits of galangal for lowering blood sugar, enhancing liver function, promoting heart health, and lowering the risk of heart disease (Kumari et al., 2015; Sutrisno et al., 2017).

The rhizomes and leaves of galangal plants frequently have high chemical contents (Arambewela et al., 2000). The rhizome of galangal contains a variety of chemical substances, including sesquiterpenes, flavonoids, phenolic acids, and essential oils (Muderawan et al., 2022). The rhizomes of galangal contain many significant chemical compounds (Tewtrakul et al., 2005; Preetha et al., 2016):

1. 1,8-cineole: an essential oil with analgesic, antiseptic, and anti-inflammatory qualities, this compound gives Kencur its unique scent.
2. Kaempferol: this flavonoid possesses anti-inflammatory and antioxidant qualities.
3. Alpinetin: this substance possesses neuroprotective, anti-inflammatory, and anti-cancer qualities.
4. Galangin: this flavonoid possesses antioxidant, anti-inflammatory, and anti-cancer qualities.
5. ethyl p-methoxycinnamate: phenolic acid with anti-inflammatory and antioxidant qualities.
6. α-humulene is a sesquiterpene compound with antibacterial, antitumor, and anti-inflammatory qualities.
7. β-sitosterol: a sterol with heart health-promoting, anti-inflammatory, and anti-cancer qualities.

K. Galanga, which is additionally referred to as Kencur, is a kind of plant that is a significant medicinal herb for Asian people, particularly those in Indonesia. It is a member of the Zingiberaceae family. While Kencur is commonly found in Indonesia as a cooking spice and as an ingredient in herbal medicine known as nasi Kencur, it is also used in India to make Ayurvedic medicines, perfumes, and cosmetics. The presence of bioactive compounds in galangal, particularly in the essential oil, is linked to its traditional medicinal use. Numerous factors, including location, source, organ, and distillation method, can affect the variations in the essential oil content of Kencur (Raina et al., 2015).

Benefits of Kencur Rhizomes

Antioxidant substances discovered in galangal rhizomes can aid in shielding bodily cells from harm brought on by free radicals. The rhizomes of galangal plants contain various antioxidant compounds, such as kaempferol, galangin, 1,8-cineole, ethyl p-methoxycinnamate, and α-humulen. High antioxidant activity was discovered in the ethanol extract from the galangal rhizome, according to research by Sulaiman et al. (2011). Using the 1,1-diphenyl-2-picrylhydrazyl test method, this study demonstrated that the ethanol extract of Kencur had an IC50 value of 7.8μg mL-1, which is the concentration required to inhibit free radical activity by 50%. Galangal rhizome has the potential to contain antioxidant compounds, which can help safeguard the body from damage caused by free radicals, according to the findings of this study. The benefits of the various bioactive ingredients in the extract of galangal rhizome are as follows:

Anti-Bacterial

Ethyl p-methoxycinnamate (EPMC), an anti-tuberculosis molecule, is produced by galangal. It has been demonstrated that galangal rhizome exhibits antibacterial activity against a variety of harmful bacterial species (Umar et al., 2012). A number of the active ingredients in Kencur, including 1,8-cineole, α-pinene, and β-pinene, are well known for their potent antibacterial properties. Water extract from galangal rhizomes has antibacterial activity against a variety of pathogenic bacteria, including *Salmonella typhimurium*, *Staphylococcus aureus*, and *Escherichia coli*, according to research by Dzoyem et al. (2019). This study assessed the antibacterial activity of galangal rhizomes using liquid dilution and agar diffusion methods.

Anti-Inflammatory

In traditional medicine, galangal has long been used to treat a variety of inflammatory conditions, including bronchitis, osteoarthritis, and rheumatoid arthritis (Khan et al., 2021; Aljobair, 2022). Kencur has also been demonstrated in numerous studies to have the ability to lower cellular inflammation. Significant anti-inflammatory activity is exhibited by the compound ethyl-p-methocycinnamate, which was extracted from isolated galangal rhizomes (Umar et al., 2011). In mice with arthritis, ethanol extract from galangal exhibited anti-inflammatory properties, according to research by Kim et al. (2015). This study demonstrates that the pro-inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), which are produced by inflammatory cells, can be reduced by galangal extract. Additional research findings regarding the anti-inflammatory properties of galangal rhizome are as follows (Table 1):

Anti-Hypertension

Numerous investigations have demonstrated galangal's potential as an antihypertensive medication and as a natural ingredient that can help lower blood pressure. Flavonoids, gingerol, and diarylheptanoid—active ingredients in galangal—have been shown to have antihypertensive properties. In rats with hypertension, ethanol extract from galangal can lower blood pressure in both the systolic and diastolic phases, according to a study by Samodra and Febrina (2020). According to this study, galangal extract can enhance nitric oxide synthase (NOS) activity and boost nitric oxide (NO) production, both of which can lower blood pressure and improve blood circulation.

Table 1: Experimental Research on the Analgesic-Anti-Inflammatory Activity of Galangal Rhizomes

No	Sample	Subject	Result	Reference
1	Ethanol extract of Kencur rhizome	Female rats induced by carrageenan	Ethanol extract of Kencur rhizome at doses of 45, 90, and 180 mg/kg significantly reduced rat edema	Samodra and Febrina (2020)
2	Chloroform extract, ethanol extract, water extract of Kencur rhizome	1% aqueous solution of bovine serum albumin	Chloroform, ethanol, and water extracts of Kencur at doses of 100-500 µg/ml have anti-inflammatory potential with protein denaturation inhibition percentage > 20%	Khan et al. (2021)
3	96% ethanol extract plaster	Male Wistar rats induced by 1% carrageenan	Ethanol extract plaster of Kencur rhizome at a dose of 45 mg/kg BW significantly reduced rat edema volume	Riasari et al. (2016)
4	Petroleum ether, ethyl acetate, and alcohol extracts	Male Sprague-Dawley rats induced by carrageenan	Petroleum ether extract at a dose of 300 mg/kg from K. galanga rhizome was found effective and significant against acute and chronic inflammation in rats	Agadish et al. (2016)
5	Diarylheptanoids 1, 2, 5, 6	RAW 2647 cells	Diarylheptanoids isolates 1, 2, 5, and 6 showed strong nitric oxide (NO) inhibition activity (IC50 17 to 46 µM) in RAW 2647 cells	Yao et al. (2018)
6	Etil p-methoxycinnamate	*In vivo*: Sprague Dawley rats (SD); *In vitro*: Anti-inflammatory test against COX 1 and COX 2	*In vivo*: EPMC at doses of 100, 200, 400, and 800 mg/kg reduced edema volume. The effect of EPMC at 800 mg/kg was not different from indomethacin; *In vitro*: EPMC at a dose of 200 µg/mL inhibited cyclooxygenase 1 (COX-1) and 2 (COX-2) enzymes by 42,9% and 57,82%, respectively	Umar et al. (2012)
7	Ethanol extract cream	Male Wistar rats	Ethanol extract cream of Kencur rhizome 3% significantly reduced edema on rat paw	Indrawati and Winda (2020)

Table 2: Effect of Various Growth Regulatory Substances on in vitro Galangal Cultivation

No	Explant	Medium and Concentration of Growth Regulators	Response	Source
1	Rhizome tip and lateral shoots	MS+2.0 mg/l BA+0.2 mg/l NAA	Callus organogenesis shoot regeneration	Anbazhagan et al. (2015)
2	Shoot buds	MS+4 mg/l BA	Plantlet production	Bhattacharya and Sen (2013)
3	Rhizome	8.87 µM BA+2.46 µM IBA+11.7 µM AgNO3	Rhizome and shoot growth	Chithra et al. (2005)
4	Rhizome tip and lateral shoots	MS+2.0 mg/l BA+0.2 mg/l NAA	Shoot multiplication and regeneration	Kalpana and Anbazhagan (2009)
5	Leaf base	MS+1.0 mg/l Dicamba+0.5 mg/l BA	Callus induction	Preetha et al. (2008)
6	Leaf base	MS+0.5 mg/l BA+0.1 mg/l NAA	Somatic embryogenesis and shoot growth	Preetha et al. (2008)
7	Leaf base	MS+15 mg/l 2,4-D+1.0 mg/l BA	Callus induction	Rahman et al. (2004)
8	Leaf base	MS+2 mg/l BA+0.1 mg/l NAA	Somatic embryogenesis	Rahman et al. (2004)
9	Rhizome	2 mg/l BAP and 1 mg/l Kn	Plantlet multiplication	Resmi et al. (2022)
10	Rhizome	MS+44 µM BAP	Microrhizome	Chirangani et al. (2005)
11	Rhizome and leaf	MS+25 mg/l IAA+2 mg/l BAP and MS+25 mg/l 2,4-D+0.5 mg/l Kinetin	Callus induction nd rooting	Swapna et al. (2004)
12	Shoot buds	MS+0.5 mgl-1 NAA+1.0 mgl-1 BA	Multiplication	Preetha et al. (2021)
13	Rhizome	MS+5 mg/l BA+30 g/l Sucrose	Plantlet production	Bhatt et al. (2012)
14	Rhizome	MS+0.25 mg/l BAP MS+0.5 mg/l BAP and MS+2 mg/l Kinetin+1 mg/l NAA	Plantlet multiplication	Ibemhal et al. (2012)
15	Rhizome	2 mg/l NAA + 12-h photoperiod	Callus induction	Shofiyani et al. (2023)
16	Shoot buds	0.3 mgl-1 BA and 0.5 mgl-1 NAA	Microrhizome	Vidya et al. (2021)

Development of In-Vitro Production of Kencur Secondary Metabolites

A common rhizome plant in Indonesia, galangal is used as a raw material for foods and drinks, spices, cosmetics, traditional medicine (jamu), and phytopharmaceuticals (Adianingsih et al., 2023). Based on empirical evidence, *K. galanga* L has been found to be effective in treating digestive issues, colds, chest and stomach pain, diabetes mellitus (Jagadish et al., 2010), antihypertensive and larvicidal activity (Kim et al., 2008), antinociceptive and anti-inflammatory (Sulaiman et al., 2011), anti-cancer and anti-monoamine oxidase (Tewtrakul et al., 2005), sedative activity (Huang et al., 2008), and anti-microbial (Kochuthressia et al., 2012). This is due to the active ingredient, saponin, flavonoids, phenols, and essential oils (Umar et al., 2011).

Tissue culture methods are acknowledged as a great resource for the propagation of medicinal plants because they enable the controlled production of contaminant-free plants that are not dependent on weather (Resmi et al., 2022). Since high-producing lines are selected, culture conditions are optimized, and precursors are supplied without causing the entire plant to grow, cell suspension cultures can produce medicinal compounds at a rate that is equal to or higher than that of intact plants (Vanisree et al., 2004). Techniques for cell suspension culture can be used not only for large-scale metabolite production but also for the investigation of significant biosynthetic pathways. According to earlier studies, axillary shoots and rhizomes were utilized as explants to develop *K. galanga* plant *in vitro* regeneration (Shirin et al., 2000).

Plant tissue culture techniques, which are *in vitro* methods, can be used to increase the number of secondary metabolites in galangal rhizomes. The content of secondary metabolites in Kencur can be increased using several *in vitro* techniques, including the following ones:

1. Callus culture: In this technique, chopped Kencur plant tissue is propagated in a nutrient medium that contains plant hormones like cytokinin and auxin. Kencur's production of secondary metabolite compounds, like EMPS, can be enhanced by callus culture (Shinde et al., 2014; Kusuma et al., 2018).
2. *In vitro* culture employing elicitors: Elicitors are compounds that can cause plants to mount defense mechanisms and produce more secondary metabolites. On Kencur, elicitors such as chitosan, salicylic acid, and jasmonic acid can be applied. The nutrient medium for Kencur tissue culture can have an elicitor added to it (Arunraj et al., 2013).
3. *In vitro* culture with illumination: Galangal plants exposed to illumination produce more secondary metabolites. This method can be done by growing Kencur

under controlled lighting conditions in an *in vitro* culture room (Srianta et al., 2019).

4. *In vitro* culture employing microorganisms: Microorganisms like fungi and bacteria can cause plants to mount defense mechanisms and produce more secondary metabolites. Kencur is grown using this method in a nutrient medium containing specific microorganisms. *Aspergillus niger* is one of the microorganisms used in the *in vitro* culture of galangal, which is used to produce phenolic compounds from galangal extract (Aziz et al., 2017). Using the *in vitro* culture method, acetylacetonate compounds are produced from Kencur extract by *Pseudomonas aeruginosa* (Nurhayati et al., 2020).

5. *In vitro* culture with growth regulators and additional nutrients: Galangal plants can produce more secondary metabolites when they are given extra nutrients like sugar, amino acids, and vitamins. Additional nutrients and PGRs can be added to the nutritional medium used for Kencur tissue culture (Kalpana and Anbazhagan, 2009).

Effect of Auxin and Cytokinin on Kencur

A chemical substance designated as a growth regulator, or PGR, is involved in controlling the growth and development of plants. Plants can benefit from PGR in several ways, including increased leaves, flowers, fruit, and root production. PGR can enhance the growth and quality of tubers in galangal plants. PGR can also strengthen galangal plants' defenses against disease and pest damage. Galangal plants are frequently treated with gibberellin, auxin, and cytokinin, among other PGR types. To prevent harm to the galangal plant and its surroundings, PGR must be used correctly and in the recommended dosages. Table 2 presents several research findings on the effect of various PGRs on Kencur in vitro.

PGR growth regulators have an impact on galangal plant production. Among the potential effects are the following:

1. Enhanced root growth: PGRs, like auxin, can promote root development and improve galangal plants' ability to absorb nutrients. Strong root systems enable Kencur plants to absorb more nutrients from the soil, which can increase plant production.
2. Increased production of rhizomes: PGRs like cytokinins can cause galangal plants to produce more tubers, which may increase the Kencur plant's tuber yield and quality.
3. Enhance the rhizome's quality: PGRs like gibberellins can make the tubers bigger and contain more active compounds in the galangal plant. This can raise the Kencur plant's economic value and enhance the quality of the tubers.

The dosage, kind, and environmental factors of the Kencur plant growth environment all affect the impact of PGR on productivity. One kind of PGR that is extremely important to a plant's growth and development is auxin. Plant hormone auxin plays a critical role in controlling the growth and development of many types of plants, including galangal plants. Auxin can affect how secondary metabolites, such as galangal, are made and accumulate in plants. Numerous investigations demonstrate that auxin administration can enhance Kencur's generation of secondary metabolites, which include substances like turmerone, xanthorrhizol, and α-farnesen. Auxin can also affect metabolic pathways and gene expression that are involved in the Kencur secondary metabolite biosynthesis process. However, the effect of auxin on the content of secondary metabolites in Kencur can vary depending on the dose and type of auxin used, as well as growth conditions and the environment in which it grows. Auxin also contributes significantly to the acceleration of apical cell growth and division. This particular hormone is also crucial for the development of the callus. Synthetic auxin groups, like NAA, are resistant to enzymatic oxidation reactions and heating during sterilization. NAA can be administered at low concentrations in *in vitro* media (Harahap, 2011). According to Matsuoka & Kokichi (1979), the concentration at which a callus forms determines its formation. Auxin can also cause callus at lower concentrations; at 0.8 mg L^{-1}, for instance it can cause callus cultures to grow on eggplant plant leaves (*Solanum melongena* L.).

Concurrently, the adenine group is the source of cytokinins, a class of chemicals. Since cytokinins are primarily involved in cell division, this activity serves as the primary factor in dividing cytokinin types. Because cytokinin and adenine, a component of DNA and RNA, have similar structures, the effect of administering this type of cytokinin is related to the level of protein synthesis. Among the cytokinin hormones that can cause calluses to form is BAP (6-Benzyl Amino Purine). One kind of cytokinin that resembles kinetin in structure is called BAP. Thus, the BAP hormone can promote the development of calluses (Harahap, 2011). Yasuda et al. (1985) reported that at a concentration of 5 µM, BAP administration could cause callus growth in *in vitro* cultures of coffee plant cotyledons (Coffea arabica). Cytokinins play a major role in the initiation of shoots from nodule segments, as explants in cytokinin-free medium do not react. Sharma et al. (1993) demonstrated that cytokinin is the primary growth regulator for shoot propagation in many medicinal plants, including Gentiana kurroo. In this study, the media containing BAP functioned as a trigger to initiate the multiplication of nodule explants. These observations are in agreement with *Swertia chirayita* (Joshi and Dhawan, 2007), *Swertia chirata* (Wawrosch et al., 1999), *Exacum affine* (Veneta et al., 2005) and *Feronia limonia* (Hossain et al., 1994), where BAP was able to increase significantly the number of shoots.

Effect of Auxin and Cytokinin on Secondary Metabolite Production in Kencur *In Vitro*

One endeavor to enhance the generation of secondary plant metabolites involves the application of biotechnological techniques like callus culture. Marchev et al. (2014) state that *in vitro* culture is an alternate technique for obtaining more secondary metabolites from the parent plant because it can alter the secondary metabolite synthesis pathway by increasing phytochemical production in plants. According to Bourgaud et al. (2001)

and Saraswati (2012), secondary metabolites are organic compounds derived from plants with a variety of structures and bioactive properties. In the context of creating medicinal compounds derived from medicinal plants, biotechnology is currently being utilized in genetic engineering, plant cultivation, and screening for endophytic microbes that can produce secondary metabolites. The utilization of biotechnological methods, particularly *in vitro* culture, is crucial in the extraction of significant plant compounds possessing chemical properties useful in medicine. Callus culture, root culture, and hair root production through transformation are among the techniques that can be developed (Rao and Ravishankar, 2002). When natural resources are scarce, the commercial production of high-value plant secondary metabolites can be achieved effectively through the use of plant cell tissue and organ culture (Vanisree et al., 2004). Extensive efforts were undertaken to identify high-yielding individuals and to optimize the selection of culture media, compositional enrichment, and organogenesis in order to enhance *in vitro* alkaloid production (Padmanabha et al., 2006; Ramesha et al., 2008; Tejavathi et al., 2012).

The success of tissue culture techniques for the production of secondary metabolites is determined by several factors, encompassing the culture media and explants used. There are several methods for boosting the manufacturing of secondary metabolites, such as the use of elicitors (Bourgaud et al., 2001), which involve subjecting the culture to chemical and physical stress to stimulate the synthesis of secondary metabolites. Protein extracts and chitosan are examples of cationic elicitors that can be applied. The growing environment's pH, UV light, and temperature control are examples of abiotic stresses that can be employed. According to Loganathan and Bai (2014), stress responses such as the use of elicitors, precursors, and biotransformation, as well as modifications to environmental factors and medium constituents, are all able to be utilized to enhance the yield of secondary metabolites under *in vitro* conditions. The following are some benefits of producing secondary metabolite compounds using plant tissue culture techniques: 1) The production system can be regulated so that production can occur when needed and in the desired quantity, approaching actual market conditions; 2) It can be done continuously, regardless of environmental factors like climate, pests and diseases, geographical obstacles, and season; 3) The quality and results of the products are more consistent; and 4) Reducing land use for these purposes (Paek et al. 2005).

Growth regulators are one of the primary external elements that support explant growth (Wahyuni et al., 2020). Because they have a genuine impact, growth regulators (PGR) play a significant role in tissue culture (Budi, 2020). When applied in small amounts, PGR, an organic compound without nutrients, can have an impact on the growth and development of plants. Auxin and cytokinin groups are PGRs that are frequently employed in tissue culture (Hariadi et al., 2019). One of the elements that determines the success of callus culture is the appropriate combination of growth regulators. According to George and Sherrington (1984), three different kinds of growth regulators are required to cause cell division: the auxin group, which includes IAA, IBA, NAA, and 2.4 D; the cytokinin and adenine group, which includes BA, BAP, DMAA, Ad-SO4, and kinetin; and the gibberellin group, which includes GA3. The appropriate kind and concentration of growth regulators can be used to promote the formation of calluses. Several variables, including growth regulator concentration, media composition, and culture duration, can cause somaclonal variation (Kour et al., 2014).

Callus culture has been developed to produce several important secondary metabolite products, incorporating vasine isolated from *Adhatoda vasica* through callus culture using MS + 2.2µM BAP + 10.7µM NAA media from petiole and leaf explants. The results showed that 90% produced callus on the 7th day, with a secondary metabolite concentration of 3.2% (Shalaka & Sandhya 2009). This is based on the results of research by Duangporn & Premjet (2009), the combination of 2mg L^{-1} NAA with 0.5mg L^{-1} BA can produce callus metabolites by producing *Phyllanthusol* compounds in *Phyllanthus acidus* plants. In line with the statement of Zhao et al. (2005), some shoot cultures were subjected to elicitor treatment, which is considered to be one of the effective strategies to increase the production of secondary metabolites. Multiple cellular processes are among the regulatory principles that drive the activation of plant secondary metabolite production. When signaling molecules are present at the plasma membrane, the cell's signal transduction network is triggered. This network involves a number of transcription factors and results in the expression of biosynthetic genes that are responsible for producing secondary metabolites. According to Wink (2010), the addition of elicitors, such as sucrose, is used to increase the production of secondary metabolites by stimulating the activity of the enzyme Phenylalanine Ammonia Lyase (PAL), which is involved in the biosynthesis pathway.

The process of forming secondary metabolites, which comes after the initiation of callus formation, depends on it. The injured area of the transplant triggers the formation of a callus by causing the transplant's cells to repair their own damaged cells. In explants, the cells begin their growth process by absorbing water and nutrients from the media. This causes the cells to enlarge and divide continuously. A collection of cells that have not undergone differentiation is called a callus and is generated by continuous division. This is consistent with Xu (2018) assertion that the explant's wound conveys out multiple internal signals to the surrounding tissue to seal the wound. Early signals will affect the regenerative capacity of the explant. Growth regulators, which are involved in healing wounds and producing callus, assist in converting damaged cells (Xu, 2018), after this initial signal is sent to multiple cells, involving the mesophyll and vascular system (Utami et al., 2007). It is believed that the growth regulator 2,4 D, which is utilized in callus proliferation media, affects RNA metabolism. This metabolism regulates protein metabolism in cells and may be involved in the transcription of RNA molecules. Treatment of 2 ppm BAP and 2-4 ppm 2,4-D

produces greenish yellow callus with a loose structure on Musa paradisiaca (Marlin & Hermansyah 2012).

A variety of secondary metabolites have been produced using medicinal plant cell cultures. The primary steps in the synthesis of secondary metabolites are callus induction and subsequent cell line differentiation. Plant cells are biosynthetically totipotent, meaning that they retain all of their genetic information when in culture, allowing them to produce metabolites that are present in the parent plant (Rao and Ravishankar, 2002). Numerous chemical compounds are discovered in plants, particularly flavonoids, diarylheptanoids, phenolics, and terpenoids. Furthermore, components or extracts of *K. galanga* have been demonstrated to have anti-inflammatory, antioxidant, anti-tumor, anti-angiogenesis, and other properties. (Umar et al., 2014a, b; Wu et al., 2015; Yao et al., 2018; Srivastava et al., 2019). Numerous secondary metabolites, including terpenoids, phenolics, cyclic dipeptides, diarylheptanoids, flavonoids, polysaccharides, and essential oils, are present in *K. galanga* according to its chemical characteristics. The rhizomes of *K. galanga* produced 97 different compounds. We have tried to cover every kind of compound and structure in this article. There has been extensive research on the chemical components of essential oils for many years. They undergo GC-MS analysis after being separated via steam distillation or supercritical fluid extraction. Terpenes, aromatic compounds, hydrocarbons, and esters are the main constituents of essential oils. Esters and terpenoids, such as ethyl cinnamate, p-methoxycinnamate, pentadecane, o-selinene, borneol, and eucalyptol, are the 19 primary constituents of essential oils (Fan et al., 2005; Zhou et al., 2006; Zhang, 2007; Cui et al., 2008; Wang et al., 2009; Sutthanont et al., 2010; Liu et al., 2017; Luo et al., 2010; Yang et al., 2018). This essential oil shows various promising pharmacological and therapeutic potentials, especially ethyl cinnamate and p-methoxycinnamate (Raina and Abraham, 2016).

Naturally, a variety of environmental factors, including soil, nutrients, climate, pests, and disease, have an impact on plant growth and presence in the field, which in turn affects the production of secondary metabolites from *K. galanga* L rhizomes for industrial purposes. Employing Kalpana and Anbazhagan's *in vitro* culture technology is another way to generate secondary metabolites (2009). The *in vitro* culture techniques described by Rajasekharan et al. (2010) can be applied not only to plant propagation and conservation but also to the production of secondary metabolites of *K. galanga* L. with better results, as cultivated rhizomes yield more oil and can be used for large-scale commercial propagation for the sustainable use of essential oils (Sahoo et al., 2014).

All of the callus samples were able to produce ethyl p-methoxycinnamate, according to qualitative and quantitative measurements and identification using a TLC scanner on the EPMC content. When exposed to light, the levels of ethyl p-methoxycinnamate were higher than when exposed to dark conditions. The average EPMC content in callus under mild environmental conditions with a sucrose concentration of 20g L^{-1} in the medium gave an average value of ethyl-p methoxycinnamate concentration of 7.49g L^{-1} and with the addition of 30-40g L^{-1} sucrose increased by 2.31 to 2.39 times (Shofiyani, 2018). Flavonoids are classified as secondary metabolites under the phenolic group. They are produced by the malonic acid and shikimic acid pathways, the principal constituents of which are the end products of the glycolysis of carbohydrates. This is consistent with Held and Piechulla's (2011) findings that three products of glycolysis—glucose 6-phosphate, phosphoenol pyruvate, and pyruvate—are used in the synthesis of secondary metabolites. Each of the three plays a specific part in the synthesis of flavonoids and other phenolic secondary metabolites.

Growth regulators have been illustrated to increase the production of phenolic acids in Ruta graveolens cultures (Ekiert et al., 2009) and hypericin and pseudohypericin in *in vitro* cultures of *Hypericum hirsutum* and *Hypericum maculatum* (Coste et al., 2011). According to studies on *Gynura pseudochina*, saponin levels in *Talinum paniculatum gaertn* (Alwiyah et al., 2015) and the highest average contribution of anthocyanin levels were obtained at the light treatment intensity of Ariany et al. (2013). Growth regulators, particularly auxin, have the effect of making the enzyme phenylalanine ammonia-lyase (PAL), which generates cinnamic phenylalanine, work harder during the synthesis of flavonoids (Rahayu et al., 2003).

Conclusion

The conclusion from the literature review of this study is that *Kaempferia galanga* L. (commonly known as Kencur), a rhizome plant with high economic value, holds significant potential in the development of secondary metabolites for use in the pharmaceutical, cosmetic, and traditional medicine industries. Kencur contains various bioactive compounds such as Ethyl p-Methoxycinnamate (EPMC), which exhibit anti-inflammatory, antioxidant, and anticancer properties. However, the main challenges in Kencur cultivation include long harvesting cycles, low quality, and a lack of government support and innovation in cultivation techniques. Therefore, *in vitro* methods such as callus culture and the use of plant growth regulators (PGRs) like auxin and cytokinin present promising alternatives for enhancing the production and quality of Kencur's secondary metabolites. The study highlights that the appropriate combination and concentration of PGRs can significantly improve the production of desired secondary metabolites, such as EPMC, through *in vitro* culture. This technology not only enhances the quality and quantity of Kencur production but also reduces reliance on unstable environmental conditions, making it more consistent in meeting the needs of the pharmaceutical and cosmetic industries.

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