*Original Article*

# Callogenesis, Growth and Bioactive Compound of Kaffir Lime (*Citrus hystrix* DC.) Callus Derived from Leaf and Stem Explants

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**Abstract**

Differences in plant organs that are used as sources of explants can cause differences in callus growth and synthesized bioactive compounds. Objective of this study was to induced calli from different sources of explants and analyzed their growth and bioactive compounds in the calli from leaf and stem explants of kaffir lime seedlings. Kaffir lime seeds were germinated until they grow into seedlings. On day 35, the leaves and stems of seedlings were harvested and induced callus. Results showed that callus initiation time of stem explants were 5.66 days faster than of leaf explants which required 12.42 days. The color of both callus was slightly different. Furthermore, callus fresh weight of leaf explants was less than of stem explants and the stationary phase of leaf explant-derived callus was earlier than stem explant. Bioactive compounds detected in callus derived from leaf and stem explants were different. The main compounds found in the leaf explant-derived callus were n-Decanoic acid and Hexanedioic acid, bis (2-ethylhexyl) esters while stem explant-derived callus was n-Hexadecanoid acid. The presence of various bioactive compounds contained in these calli proves its potential as a natural medicine.

**Keywords:** In vitro seedling, callus, explant, kaffir lime, bioactive compounds

## 1. Introduction

Kaffir lime (*Citrus hystrix* DC.) has long been used as a traditional medicinal plant because it has biological activities such as antibacterial, antioxidant, antifungal, anti-inflammatory and antiviral (An et al., 2021). Previous studies have shown that kaffir lime leaf extract is cytotoxic against cervical cancer and neuroblastoma cells

(Tunjung, Cinatl, Michaelis, & Smales, 2015), and has an inhibitory effect on leukemia K526 cell line (Anuchapreeda et al., 2020). Essential oil from the peel extract of kaffir lime fruit has antibacterial activity against Gram positive and Gram-negative bacteria (Sreepian, Sreepian, Chanthong, Mingkhwancheep, & Prathit, 2019). Kaffir lime fruit extract can inhibit the growth of *Streptococcus mutans* bacteria (Utami et al., 2020). The twigs of kaffir lime can be used as antioxidants because they have the main component of citronellal (Warsito et al., 2017 in Silalahi, 2020). Thus, each part of kaffir lime plant has a different bioactive content and biological activities that can be used as traditional medicine.

Several studies showed biosynthesis of the secondary metabolites can occur in all plant parts, including in roots, leaves, shoots, flowers, fruits, and seeds (Anggraito et al., 2018). Maslakhah, Mutiah, Hakim, Aprinda, & Suryadinata (2018), reported that extracts from the roots, stems, leaves, and seeds of the *Helianthus annuus* L plant produce varying amounts of compounds. Accordingly, terpenoid compounds, such as sesquiterpenes, triterpenes, and steroids are found in the flower of *Helianthus annuus* L. extracted using methanol. Leaf extract contains compounds of the alkaloid, flavonoids, and phenols. Stem extract contains alkaloid compounds, phenols, and flavonoids. Seed extract contains carbohydrates, flavonoids, tannins, alkaloids, saponins, and essential oils. Therefore, it is necessary to produce bioactive compounds from several plant organs.

One of the strategies to produce bioactive compounds from plants on a large scale are establish callus and cell suspension cultures (Chandana, Nagaveni, Heena, Kolakar, & Lakshmana, 2018). Callus is very important in regenerating plants because each plant cell has the ability to form new plantlet (Rasud & Bustaman, 2020).

Moreover, in vitro seedling is one of the tissue culture techniques that can be used to produce a sterile source of explants. Explants obtained from in vitro seed germination can reduce failure rates in callus cultures due to contamination (Anggraeni, 2016). Each part of the seedling are meristematic properties. Hypocotyl is the longest part of seedling so that this explant is more beneficial for callus production (Setiaji, 2020). Abbas, El-Shabrawi, Soliman, and Selim (2018) successfully induced callus from various explants from in vitro germinated seedlings, namely the stems, roots and leaves of the African locust bean plant *Parkia biglobosa* (Jacq.) Bench.

A previous study by Tunjung et al., (2021) was successfully induced a friable kaffir lime callus using seed explants. The seeds are undifferentiated embryonal tissue which can be induced to be plantlet or callus. Seed is suitable to be used as an explant source because the seed is located inside the fruit, thus it is easy to avoid contamination. However, it takes a time to wait for the fruits. Considering picking ex vitro plant organs directly from the outdoor environment is prone to contamination, thus in vitro seedling is carried out to maintain the availability of explant sources under controlled conditions. The leaves and stems of the kaffir lime plantlet are used as a source of explants for callus induction because they have gone through organogenesis so the cells and tissues formed are different. Therefore, the objective of this study was to determine the callogenesis, growth and synthesis of bioactive compounds from callus initiated from in vitro seedling leaf and stem explants.

## 2. Materials and Methods

### 2.1 Materials

Fruit of kaffir lime were collected from farm at Pekuten Village, Bayan Purworejo District, Central Java, Indonesia. Only good fresh fruits at diameter

approximately 5-6 cm was used as a sample. The fruits were peeled and seeds at size of ± 0.9-1 cm in length and ± 0.4-0.5 cm in width were excised and used for in vitro germination.

**2.2 Seed germination and growth of seedling**

The basal media used was Murashige and Skoog (MS) (Murashige & Skoog, 1962) which contains macronutrients, micronutrients, iron, vitamins, myo-inositol, sucrose, and agar. The pH was adjusted to 5.8. Kaffir lime seeds were sterilized using 5.25% NaClO by shaking for 5 minutes under aseptic conditions. Then sterilized again with 70% alcohol. The sterilized seeds were inoculated into culture bottles containing solid MS media without growth regulators (MS0). Each bottle contained 4 seeds. The culture was stored and incubated in a culture room under dark conditions at a temperature of $25^0$C and a humidity of about 50% until radicle appears. Then, the culture bottle was placed in a bright state until 35-40 days with 1000 lux light intensity.

**2.3 Callus Induction and Growth Determination**

Seedling at age of 35-40 days after germination were used as source of explants for callus induction. The leaf explants were cross cut at size of ± 0.5-1 cm and the stem explants were cut into 1 cm in length. Both explants were cultured on basal solidified MS medium with plant growth regulator (PGR) 2,4-D: BAP 1:0.5 ppm from previous study (Tunjung et al., 2021) by the wound side in contact with the culture medium. The culture were incubated in a culture room under dark conditions at a temperature of 25°C.

Callus induction time was characterized by the appearance of cell masses on the wound surface of explant. Callus biomass in terms of fresh weight and dry weight were

measured every 5 days for 50 days. These data were analyzed to obtain the callus growth curve. Callus color was measured using the Royal Horticultural Society color chart whereas the texture of callus was observed by tipping the callus using forceps and made a scoring.

### 2.4 Secondary metabolite analysis

Callus derived from both leaves and stems explants was harvested in the stationary phase. The stationary phase is the optimum condition for secondary metabolite analysis because growth has stabilized (Purwaningsih et al., 2016). The callus was then dried at $37^0$C for 24 hours until it reached a constant weight. A dry sample of callus of 100 mg was dissolved in 5 ml of ethyl acetate. Maceration was carried out for 24 hours with 3 times of re-maceration. The extracts were analyzed using gas-mass spectrometry chromatography (GC-MS) on Agilent chromatography 60034. As much as 1 mL from the extracted sample was injected into the instrument. Helium was used as a mobile phase at 1 mL/min. The spectrum of components obtained was compared with the National Institute of Standards and Technology (NIST) database 17 GC Method/Retention Index Library. The relative percentage of each component of the compound was calculated by comparing the average peak area with the total area.

## 3. Results and Discussion

### 3.1 Results

#### 3.1.1 In vitro germination and growth of seedlings

Germination is characterized by the appearance of radicle from inside the seeds. All the kaffir lime seeds were successfully undergone germination (100%). The radicle

continued growing downward and hypocotyl with cotyledons grew later on upward. In this study, radicle appeared 6 days after sowing seeds whereas the cotyledon appeared on day 15. Kaffir lime seedling at age of 35-40 days producing true leaves at size of 1 cm in length and width was used as explant for callus induction. These phenomenon can be seen in Figure 1.

### 3.1.2 Callus Induction and Growth Determination

The callus initiation time was calculated when the callus first appears after the first day of induction (Table 1). The emergence of callus was characterized by the presence of swelling of the explants on the injured area accompanied by white patches.

Leaves and stems explants were 100% successfully formed callus. It is supported by Tunjung et al., (2021), that the addition of growth regulators such as 2,4-D and BAP can induce callus from seed explants. However, there was a significant difference in the timing of callus induction between leaf callus and stem callus. Table 1 showed that the initiation time of the leaf and stem explant callus were on day 12.42 and 5.66, respectively. As a comparison, our previous study showed that induced from kaffir lime seed explants took 7.78 days to form a callus (Tunjung et al., 2021).

### 3.1.3 Callus Growth Curve

The wet and dry weights of the leaves and stems explant callus were weighed to create a callus growth curve (Figure 2). The callus growth curve between leaves and stems explant had a significant difference in each phase of callus growth (Table 2). Table 2 showed the different of growth phase of callus from leaf and stem explants. The exponential phase of stem explant callus is faster compared to leaf callus explants. In addition, the stationary phase of the stem explant callus is slower than that of the leaves

explant callus. The growth and development of callus can be seen from the biomass produced. The biomass of stem explant callus is higher than that of leaves explant callus. Our previous study (Tunjung et al., 2021), showed kaffir lime callus derived from seeds explant enters the exponential phase on day 10 and the stationary phase begins on day 35. Therefore, each organ of kaffir lime had different growth phase.

Figure 2 showed the biomass of stem explant callus was higher than that of leaf explant callus. This is supported by Suhartanto, Astutik, Umami, Suseno, & Haq (2022), where the callus explant stems of the white srikandi corn plant (*Zea mays* L.) produce the highest fresh weight compared to root and leaf explants. The fresh weight of the callus indicates that cell division occurs in the callus, so a high fresh weight indicates the cells are actively dividing.

### 3.1.4 Callus Morphology

Table 3 showed the change of calli color and texture. At day 50$^{th}$ callus from leaves explant had light yellow-green color while stems explant showed brilliant yellow green color. Callus color indicates the degree of development of the formed callus. Furthermore both leaves and stems explants callus had a friable texture. The callus's score described changes in texture from the compact seed, into the callus which getting friable day by day. The friable callus is a good callus for the manufacture of suspension cultures in an effort to multiply cell mass (Yelnititis, 2012). Leaves and stem explants callus induced with ZPT 2,4:BAP (1: 0.5) is a type of callus with a friable texture. Induced callus in media with growth regulator 2,4-D: BAP (1: 0.5) has a larger size and is good for long-term storage (Tunjung, 2021).

### 3.1.5 Bioactive Compounds

Figure 3 showed compounds detected in leaves and stem were 37 and 23 peaks, respectively. The names of compounds and their biological activities were presented in Table 4 (callus from leaves explants) and Table 5 (callus from stem explants). Bioactive compounds in both calli are mostly fatty acids and their derivative. The n-Decanoic acid and Hexanedioic acid, bis (2-Ethylhexyl) esters are the 2 main components in leaves explant callus extract while the n-Hexadecanoic acid compound is the main compound component of the stem callus explant. Furthermore 2,2-Dimethyl-6-methylene-1 [3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol and Limonen-6-ol, pivalate were the common compounds detected in both stem and leaf-derived-callus.

**3.2 Discussion**

Objective of this study was to induce kaffir lime callus from several plant organs. Previously we successfully induce friable callus from seed explant. In this study, we used leaves and stems produced by in vitro seedling for explants sources. As our knowledge, this is the first study using kaffir lime plantlet for callus induction. Plantlet as a source of explants has advantages. This is supported by Umami, Respati, Rahman, Umpuch, & Gondo (2022), which state that each part of the plantlet can be induced into a callus because it has meristematic properties that the cells are still actively dividing. Study by Nursadi et al., (2003) in Purba, Yuswanti, & Astawa (2017), the use of explants or meristematic planting material can avoid browning in callus. Differentiation leads to the formation of permanent tissues of meristematic tissue that have a certain structure and function. Furthermore, this study revealed that the use of plantlets as a source of explants will produce more biomass because each part of the plantlet can be used for callus induction.

Callus initiation is characterized by the presence of swelling of the explants on the injured area accompanied by white patches (Khaniyah & Habibah, 2012). Swelling or thickening of the explant occurs due to the interaction between the explant and the planting medium, hormones, and the appropriate environment so that the explant increases in size (Rasud & Bustaman, 2020). The initiation time of the stem explant was 5.66th days, whether the leaf explant took 12.42 day. Accordingly, stem explants are faster in forming calli than leaf explants. This is in accordance with the study by Naser & Wisnu (2020), showing that the initiation time of the leaf and stem explant callus (*Chrysanthemum morifolium* Ramat cv Dewi ratih) has a different time. The leaf explant callus is formed on day 13 and the stem explant callus on day 7. Differences between explants indicate different types of organ differentiation, totipotency, and levels of endogenous auxin hormones (Tarrahi & Rezanejad, 2013).

Callus growth is characterized by an irreversible increase in biomass (I'anatushshoimah, Nurchayati,, Y, Prihastanti, E., & Hastuti, 2020). Callus biomass also depends on the morphology of the callus, the speed of cell division, and the enlargement of the callus so that the role of growth regulators is very important for callus growth (Shinta, Minarno, & Rofiqoh, 2020). In this study, the biomass of the stem explant callus was higher than that of the leaves callus, where on the $50^{th}$ day the weight of the stem callus reaches 0.05 grams while the leaves callus is only 0.03 grams. These data supported by Kartikasari, Hidayat, & Ratnasari (2013), the growth of callus in one plant species can differ depending on the growing conditions and the location of the explant part used in the plant. Furthermore Mastuti, Widoretno, & Harijati (2020), also says that different types of explants give different responses to the speed of growth and development of callus.

241 Furthermore, growth regulators such as auxins and cytokinins with balanced
242 concentrations are able to initiate cell division and optimize cell growth (Prashariska,
243 Pitoyo, & Solichatun, 2021). The auxin hormone 2,4-D is used in callus culture because
244 of its strong activity to push ahead the process of cell differentiation, suppress
245 organogenesis, and maintain callus growth (Indah & Ermavitalini, 2013). Moreover,
246 2,4-D stimulates the optimal proliferation and growth of embryogenic callus (Carsono et
247 al., 2021). BAP plays a role in spurring explant growth, cell division, and bud formation
248 (Nadeak, Anna, & Siregar, 2012). It can stimulate cell division, increase protein
249 synthesis and influence callus growth and the production of secondary metabolites.
250 Moreover, the addition of BAP to MS medium produces higher callus biomass than
251 without the addition of BAP (Tunjung et al., 2021). Callus induced in a medium with a
252 combination of auxin and cytokinin hormones has a good effect on the formation and
253 growth of callus, and also affects the production of secondary metabolites (Bienaimé et
254 al., 2015).

255 The color of both calli changed day by day until it becomes brownish-yellow.
256 The callus of the leaf explants was light yellow-green while the stem explant callus was
257 brilliant yellow-green. Callus color was influenced by various factors, such as variations
258 in the type of organ differentiation, the level of activity of endogenous hormones,
259 pigmentation, and the type of explants used (Garcia, Pacheco, Falcão, Borges, &
260 Mansur, 2011). The difference in callus color indicates the degree of callus
261 development (Royani et al., 2020). The green callus indicates that the callus cells are
262 still actively dividing and contain chlorophyll (Sinaulan, Lenkong, & Tulung 2019).
263 Green callus has a high content of bioactive compounds and has the potential as an
264 antioxidant (Ashokhan, Othman, Abd Rahim, Karsani, & Yaacob, 2020). A callus is

brown due to an increase in the accumulation of phenolics such as lignin and a decrease in peroxidase activity towards the end of the culture period. The cells on the callus continue to turn brown and die (Chaudhary & Dantu, 2015).

On the other hand, callus from leaves and stems explant have different type of bioactive compounds. This is supported by Lahsin et al., (2016), where callus from seeds, leaves and groundcherry fruit (*Physalis peruviana*) explants contain different bioactive compounds. Some secondary metabolites are unevenly distributed in the plant organs. Moreover, the expression of the secondary metabolite compounds synthesis depends on the stage of development of the organ (Anggraito et al., 2018). Young leaves on tobacco plants (*Nicotiana tabacum* L.) produce the most salicylic acid compared to adult leaves and stems and are not found in the roots (Nugroho, 2014).

According to Koperuncholan et al., (2015), bioactive compounds are generally produced on certain synthesis pathways that can differ between types of compounds and plant species. The presence of differences in the amount and type of bioactive compounds in kaffir lime leaves and stems explant callus revealed that the bioactive compounds were distributed unevenly in various organs. The bioactive compounds detected in both calli have several biological activities (table 4 & 5) such as antibacterial, antifungal, antiviral, Antioxidant, Anti-inflammatory, anticancer etc. Therefore, calli in recent study can be used as valuable source for natural medicine because different compounds in each plantlet organ can complement each other.

## 4. Conclusions

Stems explants have a shorter callus initiation time than leaves explants. The callus color and texture have differences caused by the type of explants used. The stem derived-callus biomass was higher than the leaf derived-callus but the identified

bioactive compound content was less. The compounds detected in the leaves and stem explant callus extract were mostly fatty acid groups and their derivative, namely n-Decanoic acid, Hexanedioic acid, bis (2-ethylhexyl) ester and n-Hexadecanoid etc, which have the potential to be in various biological activities.

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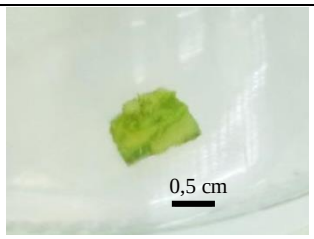
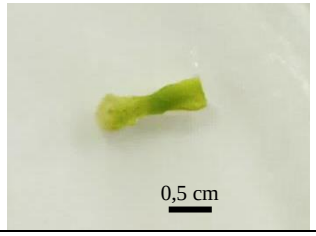
Table

**SJST MANUSCRIPT FOR A TABLE FILE**

Tabel 1. Callus Initiation Time

| Number | Types of Explants | Callus initiation time (Days) | Picture |
|---|---|---|---|
| 1. | Leaves | 12,42 ± 0,83[a] |   |
| 2. | Stems | 5,66 ± 0,29[b] |   |

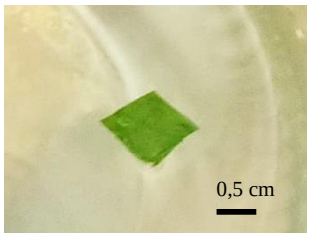
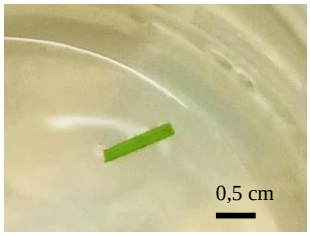
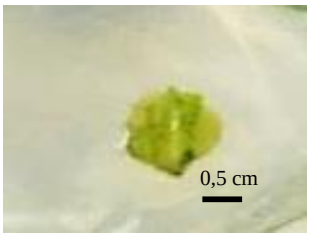
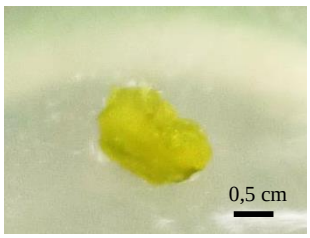
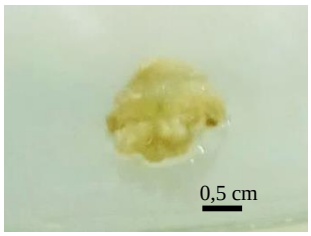
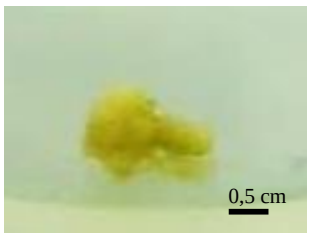
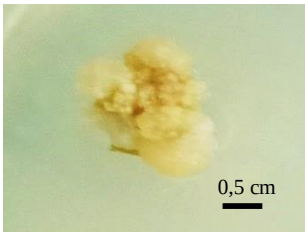
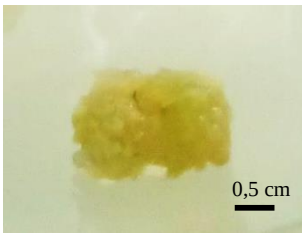
Numbers followed by different letter notation in different columns indicate a significant difference based on a one-way ANOVA analysis.

Table 1 showed that the initiation time of leaf explant callus is on day 12.42 and stem explant callus is on day 5.66. This is in accordance with the research by Naser & Wisnu (2020), showing that the initiation time of the leaf and stem explant callus (*Chrysanthemum morifolium* Ramat cv Dewi ratih) was different. Leaf formed callus on day 13 whereas stem formed callus on day 7. The difference in time required for callus formation might be due to different types of organ differentiation, totipotency, and levels of endogenous auxin hormones (Tarrahi & Rezanejad, 2013).

Table 2. Growth Phase of Callus

| Explant | Growth Phase (Days) | | |
|---|---|---|---|
| | Lag phase | Exponential phase | Stationary phase |
| Leaves | 0-15 | 15-30 | 30-50 |
| Stems | 0-10 | 10-35 | 35-50 |

Table 3. The Morphology of Callus

| Days | Type of Explant | | | |
|---|---|---|---|---|
| | Leaf | | Stem | |
| 1 |  | Color : 140 A (Vivid Yellowish Green) Texture : (-) |  | Color : 141C (Strong Yellowish Green) Texture : (-) |
| 15 |  | Color : 140 B (Briliant Yellow Green) Texture : (+) |  | Color : N144B (Strong Yellow ) Texture : (+) |
| 35 |  | Color : 142 A (Strong Yellowish Green) Texture : (++) |  | Color : 149 A (Briliant Yellow Green) Texture : (++) |
| 50 |  | Color : 142 C (Light Yellow Green) Texture : (+++) |  | Color : 150 B (Brilliant Yellow Green) Texture : (+++) |

- : no callus has appeared yet
+ : the texture is compact
++ : the callus spreads to all parts and the texture is less friable
+++ : callus mass dominates explant mass and friable texture

Table 3 showed that the color and texture of callus from leaves and stem explants. Leaves explant callus has light yellow green color and stem explant callus has brilliant yellow green color. Callus color indicates the degree of development of the formed callus. Leaves and stem explants callus induced with ZPT 2,4:BAP (1: 0.5) is a type of callus with a friable texture. Friable callus is a good callus for the manufacture of suspension cultures in an effort to multiply cell mass (Yelnititis, 2012). Induced callus in media with growth regulator 2,4-D: BAP (1: 0.5) has a larger size and is good for long-term storage.

Table 4. Content of Bioactive Compound of Callus from Leaves Explant

| No | Compounds | Peak Area (%) | Retention Time (RT) | Group | Biomedical Activities |
|---|---|---|---|---|---|
| 1 | 2-Hexanol, 3,4-dimethyl | 0,26 | 4,57 | Fatty Alcohol | - |
| 2 | Octanoic acid | 1,37 | 20,12 | Saturated Fatty Acid | Insenticidal,Antimicrobial (Kaczmarek et al.,2022) |
| 3 | n-Decanoic acid | 24,1 | 28,93 | Saturated Fatty Acid | Antibacterial, Antifungal (Belakhdar et al., 2015) |
| 4 | Cyclopentanol | 0,35 | 31,2 | Alcohol | - |
| 5 | 2,4-Di-tert-butylphenol | 0,76 | 34,26 | Phenol | - |
| 6 | Oxalic acid, allyl pentadecyl ester | 0,34 | 37,59 | Dycarboxylic acid | - |
| 7 | 2-Cyclopropylbutan-2-ol | 0,33 | 37,87 | Alcohol | - |
| 8 | 4-Oxo-.beta.-isodamascol | 0,32 | 44,04 | - | - |
| 9 | 2-Piperidinone, N-[4-bromo-n-butyl]- | 0,78; 1,7 | 44,95; 51,04 | Ketone | Antimicrobial activity (Al-Bahadily et al., 2019) |
| 10 | Heptadecane, 2,6,10,14-tetramethyl- | 1,7 | 45,19 | alkane hydrocarbon | - |
| 11 | Evodinnol | 2,97; 0,3 | 47,89; 49,57 | Salicylaldehyde | - |
| 12 | 7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione | 0,53 | 48,47 | Cylic ketone | Antineoplastic, antimicrobial and antiviral activities (Tatipamula et al., 2019) |
| 13 | Undec-10-ynoic acid, tridec-2-yn-1-yl ester | 0,63 | 49,10 | Aliphatic | - |
| 14 | 1,2-Benzenedicarboxylic | 2,02 | 49,90 | - | - |

| | | | | | |
|---|---|---|---|---|---|
| | acid, butyl octyl ester | | | | |
| 15 | 2,2-Dimethyl-6-methylene-1 [3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol | 0,45; 0,27; 0,29; 0,88; 1 | 50,04; 52,87; 54,89; 56,54; 56,88 | Methyl ester | - |
| 16 | n-Hexadecanoic acid | 11,78 | 50,26 | Long chain Fatty Acid | Antioxidant, Anti-inflammatory, Antibacterial, Pestiside, cancer preventive (Hameed et al., 2015) |
| 17 | Oleic Acid | 0,72 | 50,90 | Monounsaturated omega-9 fatty acid | Antioxidant, Antifungal, Antiviral, Anti Bacterial (Dailey et al., 2011), Anticancer activity against MCF-7 and HT-29 cancer cells (Batur et al., 2019) |
| 18 | Ethanone, 1-(1,2,3,4,7,7a-hexahydro-1,4,4,5-tetramethyl-1,3a-ethano-3aH-inden-6-yl)- | 0,43 | 52,28 | Salicylaldehyde | - |
| 19 | Limonen-6-ol, pivalate | 0,25 | 53,13 | Monoterpene | Antioxidant, Anti-inflammatory, insect repellent activity (Abdulhafiz et al., 2020) |
| 20 | 2-Pentanone, 4-cyclohexylidene-3,3-diethyl- | 1,34 | 53,84 | Acyclic sesquiterpene alcohol | - |
| 21 | cis-7-Hexadecenoic acid | 11,14 | 53,96 | Monounsaturated Fatty Acid | Anti-inflammatory (Astudilo et al., 2018) |
| 22 | 7-Methyl-Z-tetradecen-1-ol acetate | 1,22; 1,15; 0,51; 0,39; 1,61; 0,25 | 54,45; 54,99; 55,37; 56,78; 57,65;58,16 | Acetate ester | Anticancer, Anti-inflammatory, Hepatoprotective (Hameed et al., 2015) |
| 23 | Eeicosatetraenoic acid | 0,4 | 55,34 | Polyunsaturated Fatty Acid | Antimicrobial, Antibacterial (Thien le et al., 2017) |
| 24 | 9,12,15-Octadecatrienoic acid | 1,45 | 57,62 | Polyunsaturated Fatty Acid | Anti-inflammatory (Rigoberto et al., 2017) |
| 25 | Hexanedioic acid, bis(2-ethylhexyl) ester | 22,78 | 57,98 | Long chain fatty acid | Antioxidant, Anti-inflammatory, Antibacterial, Pestiside, cancer preventive (Hameed et al., 2015) |
| 26 | Sulfurous acid, cyclohexylmethyl octadecyl ester | 3,19 | 58,25 | Long chain fatty acid | Pesticide activity (Ravi et al., 2018) |

: compounds that have the highest percent peak area
: compounds found in leaf callus and stem callus

Table 4 showed the bioactive compounds detected in callus from leaves explants. The n-Decanoic acid and Hexanedioic acid, bis (2-Ethylhexyl) esters are the 2 main components in leaves explant callus extract.

Table 5. Content of Bioactive Compound of Callus from Stems Explant

| No | Compounds | Peak Area (%) | Retention Time (RT) | Group | Biomedical Activities |
|---|---|---|---|---|---|
| 1 | 1,8-Nonadien-3-ol | 1,71 | 3,11 | Fatty Alcohol | Antioxidant potential, Antibacterial (Muthumperumal et al., 2016) |
| 2 | Octanoic acid | 1,13 | 20,20 | Saturated Fatty Acid | Insenticidal activity, Antimicrobial (Kaczmarek et al., 2022) |
| 3 | n-Decanoic acid | 3,74 | 28,97 | Saturated Fatty Acid | Antibacterial, Antifungal (Belakhdar et al., 2015) |
| 4 | Tetradecane, 1-chloro | 0,85 | 47,89 | Ester | Antioxidant and anti-inflammatory (Palariya et al., 2019) |
| 5 | n-Hexadecanoic acid | 41,35 | 50,34 | Long chain Fatty Acid | Antioxidant, nematicide, pesticide, Hypocholesterolamic (Siswadi et al., 2021) |
| 6 | 2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol) | 0,85; 2,69; 2,21; 0,62, 1,1; 1,36; 4,09; 1,98; 0,61; 0,68 | 52,29; 54,86; 55,56; 55,84; 56,25; 56,54; 57,03; 57,67; 58,08; 58,15 | Methyl ester | - |
| 7 | Z-8-Methyl-9-tetradecenoic acid | 0,48 | 52,96 | Fatty Acid | Antibacterial activity (Kadhim et al., 2016) |
| 8 | Tetradecane, 2,6,10-trimethyl | 1,77 | 53,1 | Fatty Acid | Antimicrobial (Sheoran et al., 2015) |
| 9 | cis-Vaccenic acid | 8,83; 7,12 | 54,03; 54,12 | Fatty Acid | Antibacterial activity and hypolipidemic effect in rat (Semwal et al., 2018) |
| 10 | Octadecanoic acid | 8,67 | 54,48 | Stearic Acid | - |
| 11 | 9,12,15-Octadecatrienoic acid | 1,11 | 54,97 | Polyunsaturated Fatty Acid | Anti-inflammatory (Rigoberto et al., 2017) |
| 12 | Limonen-6-ol, pivalate | 1,11 | 56,62 | Monoterpene | Antioxidant, Anti-inflammatory (Abdulhafiz et al., 2020), Insect repellent activity (Mohiuddin et al., 2018) |
| 13 | Adipic acid | 5,93 | 57,98 | Long chain fatty acid | Antioxidant, nematicide, pesticide, Hypocholesterolamic (Siswadi et al., 2021) |

: compounds that have the highest percent peak area
: compounds found in leaf callus and stem callus

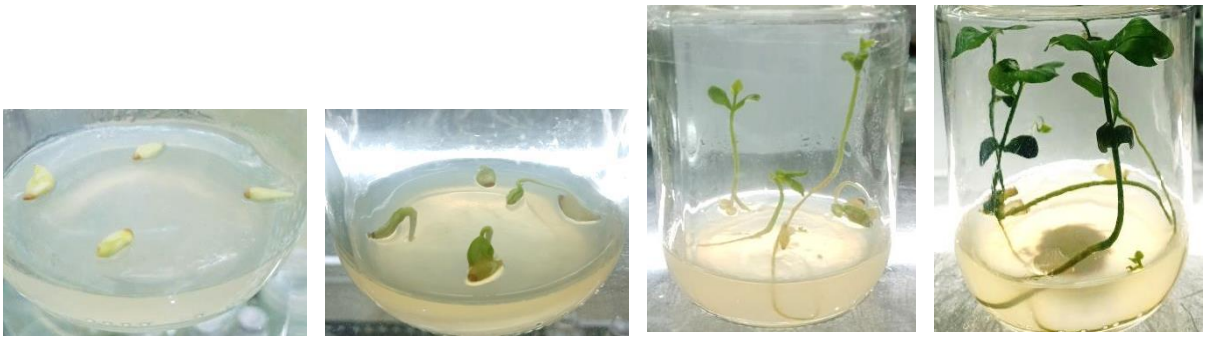
Table 5 showed the bioactive compounds present in callus from stem explants. The n-Hexadecanoic acid compound is the main compound component of the stem callus explant.

Figure

**SJST MANUSCRIPT FOR A FIGURE FILE**

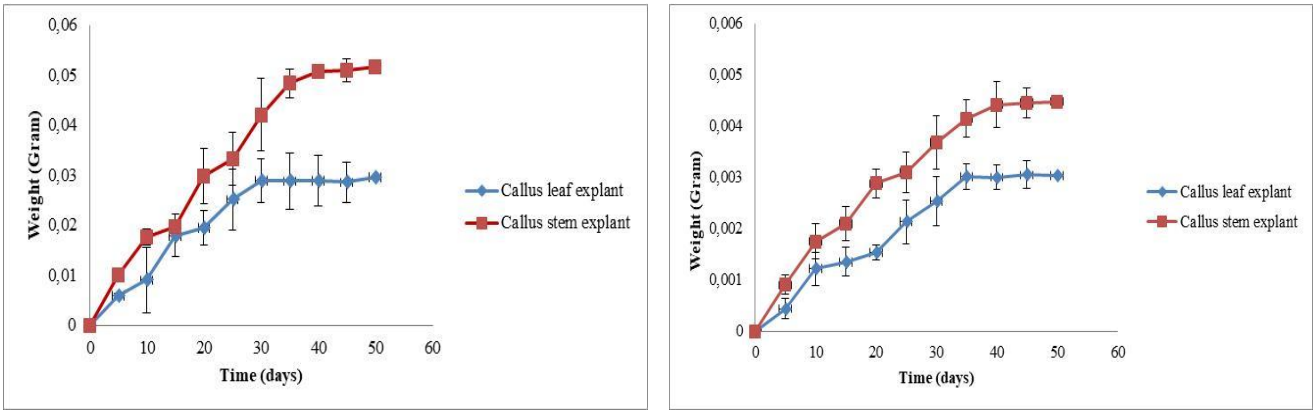
## List of Figure





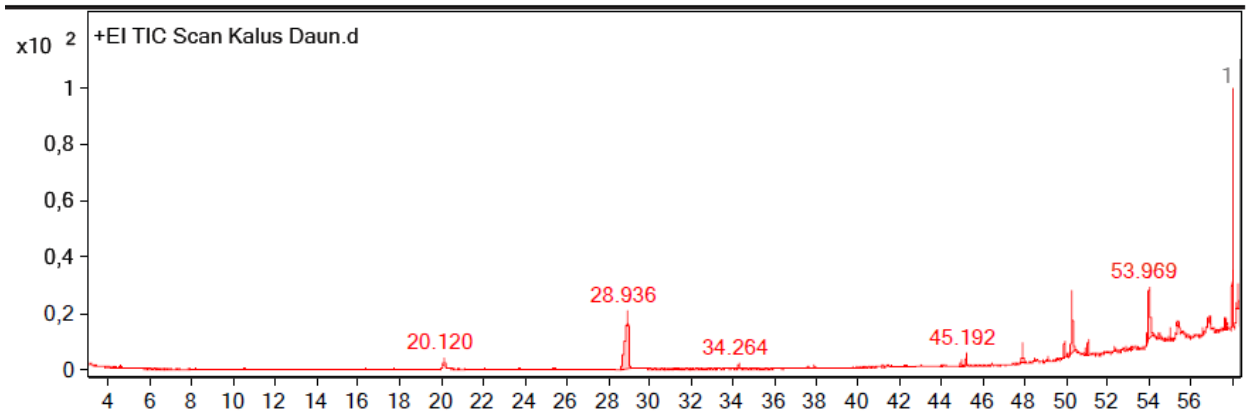
(a) (b) (c) (d)

Figure 1. Germination of kaffir lime. (a) 0 day (b) 6 day (c) 15 day (d) 35 day

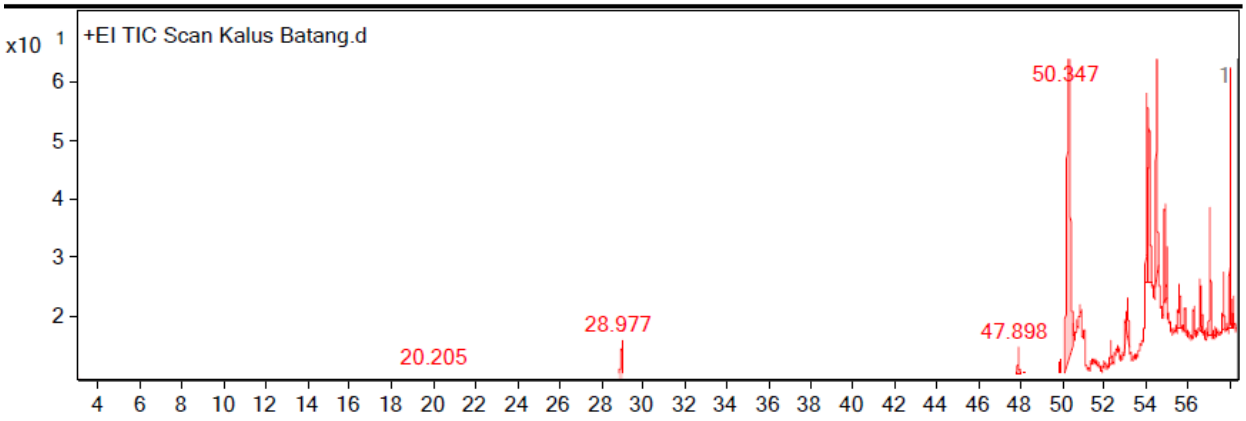



(a) (b)

Figure 2. Growth curve of callus from leaves and stem explants. (a) Gross weight (b) Dry weight




(a)




(b)

Figure 3. TIC of Callus Extract. (a) Callus From Leaves Explant (b) Callus From Stems Explant