RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2023.044

ScienceAsia 49 (2023): **507–514**

Cell line screening and characterization of bioactive compounds from kaffir lime (*Citrus hystrix* DC.) cell suspension elicited by yeast and isopentenyl pyrophosphate

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Received 11 Sep 2022, Accepted 11 Mar 2023
Available online 11 Jun 2023

ABSTRACT: Elicitation and precursor feeding are two of available strategies for enhancing bioactive production in plant cell line screening. This study aimed to select the optimum kaffir lime cell line, which yields more bioactive constituents, using elicitation and precursor feeding and analyze its bioactive compounds. Firstly, we induced callus from the seed explant. The callus tissues were, then, subjected to the cell suspension. As much as 10 mg/ml of pure culture *S. cerevisiae* (SC), 5 mg/ml (F5), and 10 mg/ml (F10) of commercial technical-grade yeast were used as elicitors. Furthermore, 10 μg/ml of Isopentenyl pyrophosphate (IPP) was used as a precursor. After obtaining the cell lines, we analyzed the profile of the bioactive compounds by GC-MS. The results showed that the number of living kaffir lime cells after treatments decreased by 37.1% to 40.4%, indicating a successful cell line selection. The resistant cells were assumed to be the cell lines capable of producing a higher concentration of target compounds (terpenoids). Bioactive compounds detected in all treatments were dominated by alkane, fatty acid, and aldehyde compounds. F5 and SC treatment enhanced more terpenoids compounds compared with untreated cells. It can be concluded that all treatments indicated a successful cell line selection, in which F5 and SC enhanced more terpenoids compared with the control. Terpenoid-producing cell lines can be used as a raw material for cancer medicine.

KEYWORDS: kaffir lime cell line, elicitor, precursor feedings, terpenoids, yeast

INTRODUCTION

As reported in our previous study, kaffir lime leaf extract is cytotoxic against cervical cancer and neuroblastoma cell lines [1]. However, the utilization of the extract obtained primarily by harvesting many kaffir lime leaves might be subjected to overexploitation issues. According to the other case, occurred mass exploitation of natural habitat of some herbal and aromatic plants is dangerous to medicinal plants, especially endemic species [2]. Moreover, harvesting leaves from nature is strongly influenced by environmental conditions. In addition to leaf extraction, plant tissue culture, especially the cell suspension culture technique, can be employed as an alternate solution to mass-producing bioactive compounds [3].

Our research group has successfully induced friable callus tissues derived from kaffir lime seeds through tissue culture techniques [4, 5]. We showed that these calluses had antibacterial activity [6]. However, the extract of kaffir lime callus derived from seed explant was not toxic to the T47D breast cancer cell line [7], whereas the callus was used as a raw material for cell suspensions. Therefore, the cell suspension must be treated to increase bioactive compounds and cytotoxicity against T47D cells. The treatments were elicitation and precursor feeding. Elicitation uses the elicitor, which acts as a signal. These signals are recognized by elicitor-specific receptors on the plant cell membrane and stimulate defense responses during elicitation resulting in increased synthesis of bioactive compounds. For the effectiveness of elicitation, it is important to optimize various parameters, such as elicitor type, concentration, duration of exposure, and timing; to give treatment. Furthermore, the combined application of various elicitors, integration with precursor feeding, or replenishment of medium could improve the accumulation of secondary metabolites due to their synergistic effect [8].

Previous studies showed that cell suspension culture could enhance artemisinin production by feeding the precursors and eliciting [3]. Both elicitation and precursor feeding improve secondary metabolite production effectively in plant cell suspension cultures of *Vitis vinifera* [9]. Furthermore, the production of any secondary metabolite from plant cells also depends on the selected cell lines [3]. Plant cell suspension culture often causes genetic instability in the growth and production of bioactive compounds due to somaclonal variation. Therefore, the selection of highly productive lines of cell suspension is important.

Recent reports of anticancer drugs derived from

natural products have led to the identification of various terpenoids that inhibit cancer cells [10]. Therefore, the production of terpenoid compounds was the target of our study. *Saccharomyces cerevisiae* has shown a positive response regarding gymnemic acid (triterpenoid glycosides) in the suspension culture of *Gymnema Sylvestre* [11]. In this study, we used yeast (either pure culture of *S. cerevisiae* or commercial technical-grade yeast). The commercial technical-grade yeast that contains natural *S. cerevisiae* is easier and more practical than pure *S. cerevisiae* culture because it is easy to search out, freely sold, cost-effective, and handy to hold around with tightly closed packaging [12], indicating the potential of commercial technical grade to be used as an elicitor. On the other hand, all terpenoids are synthesized from two types of precursors, namely, isopentenyl pyrophosphate (IPP) and dimethyl pyrophosphate (DMAPP) [13]. As the building blocks for terpenoids, enough supply of IPP and DMAPP is required for efficient terpenoid biosynthesis [13]. We, therefore, used IPP as a means of selecting those cells which could change the IPP into terpenoid compounds.

The parameters to indicate successful cell line selection included the decrease in cell quantities and the presence of damaged cells after treatment. The resistant cells were assumed to be the cell lines capable of producing a higher concentration of target compounds [14]. A previous study showed that a high concentration of pimelic acid was toxic to plant cells. Therefore, pimelic acid was used as the precursor for selecting cells that produce more biotin. Resistant cells to pimelic acid with a high level of biotin were considered a cell line [14]. In addition, according to the previous study, high concentrations of IPP treatment caused growth inhibition and reduced cell viability in *Escherichia coli*. However, with the decreased concentration of IPP, *E. coli* appeared to recover from these perturbations [15]. The present study aimed to select the kaffir lime cell lines which yielded more bioactive constituents using yeast elicitation and precursor feeding and to analyze its bioactive compounds. The novelty of this study was using yeast, either as a pure culture of *S. cerevisiae* or technical grade yeast; and IPP precursor to screen kaffir lime cells derived from seed explants. These cell lines could be used as the raw material of traditional medicines for cancer treatment.

MATERIALS AND METHODS

Plant material

Kaffir lime (*C. hystrix*) fruits were collected from Kaliduren Village, Borobudur, Magelang, Central Java. The sample was identified by Prof. Dr. Ratna Susandarini, a botanist from the Lab of Plant Systematic, Faculty of Biology, Universitas Gadjah Mada.

Callus and cell suspension induction

Callus induction, cell suspension, and determination of the growth curve of cell suspension were previously described [12, 16]. Murashige and Skoog (MS) medium, in combination with 2 ppm auxin (2,4-D) as a plant growth regulator, was used as a basal medium to induce friable callus production. Kaffir lime seeds were taken from ripe fruits. The fruits were selected based on the external morphology: uniform size, similar color, and not rotten. The seeds were sterilized with 5.25% Sodium Hypochlorite (NaClO) in the Laminar Air Flow (LAF), split and grown in a culture bottle containing treatment media. The culture bottle was sealed and kept in the dark at 20 °C for 30 days. The generation 0 (G0) callus was a subculture in the early stationary phase in the same medium, and generation 1 (G1) callus was grown under the same condition and subjected to the next experiment.

G1 Callus (10 g) in the early stationary phase (30 days old) was used as a raw material of 50 ml suspension culture. The callus was grown in Erlenmeyer flasks filled with MS liquid medium, having the same combination of plant growth regulators as in callus medium (2 ppm of 2,4-D) for 21 days. After then, the homogenate was transferred into a new liquid medium to establish cell suspension culture. The flasks were placed on a shaker with a speed of 90 rpm and ±3 cm orbital motion. The culture was incubated at room temperature in the dark. The growth curve of cell suspension was determined by calculating cells under a microscope with a hemocytometer. The calculation was carried out once every two days for 24 days. Approximately 1 ml of cell suspension was taken aseptically, and cells were counted under a microscope. The growth curve was used to determine the time for adding the elicitor and precursor.

Screening of cell line by elicitation

As previously reported, pure culture and technical grade yeast had the same morphology and growth curve [12]. One pick of inoculation needle of lab-cultured *S. cerevisiae* and commercial technical-grade yeast culture were separately inoculated into 10 ml of Peptone Glucose Yeast Extract (PGY) liquid medium and incubated at room temperature without agitation. The yeasts were harvested in the early stationary phase after 16 h of inoculation. At this time, the yeast's cell wall was completely formed. Furthermore, components of the cell wall, especially glucan, were recognized by plant cells as stress. As a result, secondary metabolites were produced in response to the stress. The media (containing yeasts) was filtered with filter paper and autoclaved. The use of dead yeast cells was to avoid contamination of kaffir lime cell suspension. After that, the yeast could be used as an elicitor. Approximately 50 ml of kaffir lime suspension culture of the early stationary phase was

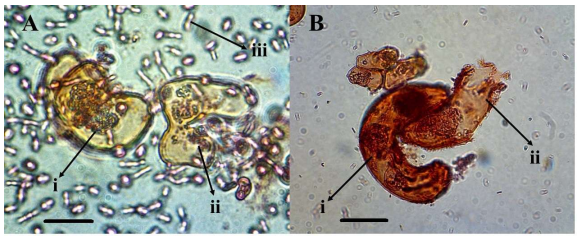

Fig. 1 Morphology of damaged kaffir lime cells after elicitation (A) and precursor feeding (B), stained with neutral red at 40 × magnification, scale bars 100 μm. The living cells (i), dead cells (ii), and yeast cells (iii) are pointed with black arrows.

mixed with 10 mg/ml of *S. cerevisiae* (SC) and two separate concentrations of commercial technical-grade instant yeast, 5 mg/ml (F5) and 10 mg/ml (F10). Elicitation treatment was carried out for four days according to the method conducted in *Azadirachta indica* cell suspension culture [17]. The morphological observation was carried out using a neutral red dye solution, and the cell counting was performed under a microscope with a hemocytometer every day for four days. The measurements were replicated three times.

Screening of cell line by precursor feeding

A total of 10 μg/ml IPP precursor was added aseptically into 10 ml of suspension culture using a microfilter (Syringe filter, PVDF Sterile, Blue, 0.22 μm) at the beginning of the lag phase. The culture was, then, incubated for three weeks. A neutral red dye solution was used in morphological observation. Meanwhile, the cell counting was performed with a hemocytometer once every two days for 24 days, a period when all phases of the growth curve of kaffir lime suspension was completed [16], Three replications were made for every measurement.

Analysis of bioactive compound

Resistant cells obtained from each treatment were filtered through a T61 nylon filter (approximate 40 μm × 40 μm pore size), when the kaffir lime cells reached 32 μm × 54 μm in size, and the size of yeast cells was about 5 μm–7 μm. The pore size of the T61 nylon filter is smaller than the kaffir lime cells but larger than the yeast cells. Moreover, kaffir lime dead cells were damaged cell and smaller than the resistant cell (Fig. 1). Fig. 1 shows the damaged cells after elicitation (1A) and precursor feeding (1B). Therefore, the yeast cells and kaffir lime dead cells could pass through the filter and be removed from the resistant cell mixture. Thus, the T61 nylon filter could separate kaffir lime cells from yeast and kaffir lime dead cells. The filtered cells were washed using a medium to ensure only kaffir lime cells remained, then, were dried in the oven at 60 °C until a constant weight was obtained. The bioactive compounds from dried cells were extracted in ethyl acetate solvent using the maceration process.

The extracts were analyzed by GC-MS Shimadzu GCMS-QP 2010S (Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia) using a nonpolar column in the form of Rtx 5 and helium gas as the mobile phase. A total of 5 μl of the extract was injected with a splitless mode injection system at an injector temperature of 300 °C. Gradient temperature, with an initial column temperature of 70 °C for 5 min, was increased to 230 °C. The detector temperature used was 300 °C, with the MS detector electron energy setting of around 70 eV. The libraries used were WILEY229 and NIST62.

RESULTS AND DISCUSSION

Screening of kaffir lime cell line by elicitation and precursor feedings

Control treatment has a stable cell count relative to other treatments. Yeast treatment was given when the cell suspension was at the beginning of the stationary phase. Phases in kaffir lime cell suspension were according to the growth curve from our previous studies [12, 16]. At the stationary phase, the cell growth achieved stability, and control cells were not affected by elicitation. Decreasing the cell number after F5, F10, and SC treatments indicated that the selection of kaffir lime cell lines was made (Fig. 2). On the first day, kaffir lime cells might have responded to the elicitor by stopping the cell division process and, instead, used the available energy to produce more bioactive compounds (mainly terpenoids) as defense mechanisms toward the elicitor. A previous study in *Linum grandiflorum* callus culture reported larger phenylpropanoid when high concentrations of yeast elicitor (500 and 1000 mg/l) were supplied in the medium compared with a control. Furthermore, the production of phenylpropanoids in *Linum grandiflorum* in vitro cultures was primarily stimulated at the earliest elicitation stages [18].

Fig. 3 shows that the cell count decreased gradually after IPP treatment from day 0 to day 4, which was opposite to the count of the control cell during the same period. The result indicated that there was a kaffir lime cell selection after the addition of IPP. This finding was supported by a previous study showing growth inhibition and reduced cell viability in *Escherichia coli* caused by high concentrations of IPP treatment [15]. Furthermore, precursor feeding at the appropriate concentrations and exposure over time could increase the production of secondary metabolites. A previous study indicated the appropriate concentration of precursor in the *Withania somnifera* (L.) Dunal culture medium capable of increasing the level of withanolides during the exponential phase and then decreased during the decline phase. [19]. This phenomenon was either caused by the suspension culture's slow growth rate or

cell lysis after 28 days.

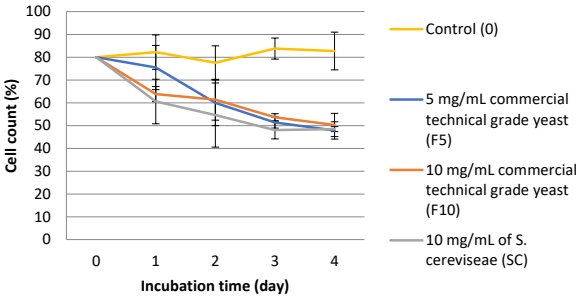

Fig. 2 The growth curve of kaffir lime cells during elicitation treatment with a pure culture and commercial technical-grade of *S. cerevisiae* yeast.

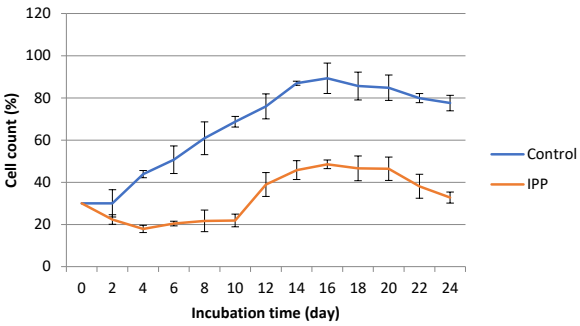

Fig. 3 Cell growth curves after IPP precursor treatment.

A 10 µg/ml concentration of IPP was quite toxic to kaffir lime cells. It damaged and killed the unresistant cells. Survival cells in this study were the resistant cells, which were assumed to be able to convert IPP precursors into terpenoids. The addition of precursors was done at the beginning of the lag phase, and there were enough nutrients left in the medium for the resistant cells to continue doubling their number. The result was supported by a study in the *Catharanthus roseus* S1 cell line, revealing that the highest terpenoid indole alkaloid (TIA) accumulation occurred when the L-tryptophan, tryptamine, loganin, and secologanin precursors were given at the inoculation time of cells into new medium (on day 0) [20]. Finally, at the beginning of the stationary phase, the nutrients in the medium started to run out, and cell lines were expected to begin producing bioactive compounds. Therefore, the cell lines with IPP precursor treatment were harvested at the stationary phase (day 20).

Fig. 1 illustrates the morphology of kaffir lime cells after being treated with elicitors and precursors. Suspension cell culture consisted of different types of cell morphology, including globular, comma, and elongated shapes. All of the three shapes were found in the cell culture every day during the treatment. Some yeast cells were detected freely in the medium, while others were attached to the wall of kaffir lime cells. Damages of plant cell wall mainly led to cell death; and, subsequently, the cytoplasm together with the organelles and nucleus would be damaged. After neutral red staining, the nucleus appeared dark red. The morphology of some cells was changed into irregular shapes.

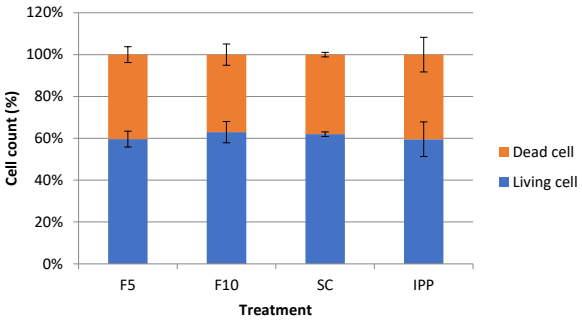

Fig. 4 Numbers of living and dead cells of cell line selection treated with F5, F10, SC, and IPP in kaffir lime cell suspension (*Citrus hystrix* DC.).

The cell line selection using F5, F10, SC, and IPP treatments showed that cell deaths occurred, and the number of living cells decreased by 37.1% to 40.4% after the treatments (Fig. 4). A decrease in living cell number indicated that the cell line selection was successfully carried out [14]. Based on the remaining viable cells, it was assumed that the resistance was a result from the ability of cells to produce more terpenoids and other bioactive compounds.

Bioactive compounds profile

The cell suspension was harvested on day 4 after the elicitation treatment, and on day 20 after the precursor treatment. The results of the bioactive analysis on the extract of harvested kaffir lime cell suspension were shown in Fig. 5.

Fig. 5 shows that each treatment produced a different number of peaks. Control treatment yielded 67 peaks, while F5, F10, SC, and IPP treatments exhibited 79, 19, 66, and 10 peaks, respectively. This variation in peak numbers indicateed that each treatment caused the cells to yield different amounts and different types of bioactive compounds. When treated with an elicitor, cells could stimulate or induce de novo synthesis of a wide range of bioactive compounds in plant cells because of their defensive reaction against attacking pathogens. For example, there was a significant enhancement of oleanolic acid accumulation by biotic elicitors in cell suspension cultures of *Calendula officinalis* [21]. The predicted bioactive compounds obtained from each treatment were shown in Table 1 and Table 2. Furthermore, bioactive compounds detected in some treatments were also compared.

In this study, 66 types of compounds were listed.

Table 1 Bioactive nonterpenoid compounds in kaffir lime cell suspension culture extracts.

No	Compound	Group	Peak area (%)				
			Control	F5	F10	SC	IPP
1	Pentadecanoic acid	Fatty acid	20.97	2.30	4.26	1.50	
2	2-Nonen-1-ol	Fatty alcohol	0.24				
3	2-Propenoic acid		0.90			0.73	
4	Octadecanal	Fatty Aldehyde	0.18	2.57		4.13	
5	Undecane	Saturated Fatty aldehyde	5.80	2.88		4.46	1.51
6	Tetradecane	Alkane	3.32	4.85	0.59	2.49	
7	N,N-Dimethyloctylamine	Fatty amine	3.69				
8	Octadecane	Fatty acid methyl ester	7.63	9.28		6.74	
9	Pentadecane	Fatty acid methyl ester	2.09	2.44		0.69	
10	9,12-Octadecadienoic acid,	Fatty acid	1.86				
11	11-Octadecenoic acid	Fatty acid	8.41				8.14
12	Dodecane	Saturated fatty acid	2.43	3.03			
13	Hexadecane	Alkane	2.89	0.41	0.50		
14	Hexacosane	Saturated hydrocarbon	1.91	2.90		0.37	
15	Heptadecane	Fatty acid	0.39				
16	Isooctane	Trimethyl Alkane	0.86		0.87		
17	Colchifoleine	Flavonoid	0.21				
18	Docosane	Alkane acyclic	1.18				
19	Pentanone	Alkyl Ketone	3.20				
20	Cholesta-8,24-dien-3-ol	Steroid	1.84	0.27	0.68	5.52	
21	Acetic Acid	Fatty acid	0.73				
22	Pentane	Fatty acid	29.08	19.27	22.21	11.21	
23	1-Dodecene	Long chain Alkene		0.28		0.32	
24	Dodecanal	Fatty aldehyde		0.36		0.77	
25	Hexadecanoic acid/Palmitic acid	Fatty ester		12.17			11.78
26	Cyclopropanepentanoic acid	Fatty acid		0.82			
27	Butane	Alkane		0.16			
28	Cyclohexane	Fatty acid		0.48			
29	N,N -Dimethyl-Tridecylamine			6.40			
30	Hexanedioic acid/adipic acid	Fatty acid ester		0.46	0.91	0.43	
31	Ethene	Simple aliphatic terminal alkene		0.67			
32	1-octanol	Fatty alcohol		4.64		2.56	
33	Octadecanone	Aliphatic linear ketone		0.45			
34	Tetratetracontane	Fatty acid methyl ester		1.32			
35	Octadecanoic acid	Fatty acid		1.39	29.31	31.03	
36	1,2-Octadecanediol			0.42			
37	Decane	Alkane		10.61			
38	Lauric acid	Fatty acid		0.91			
39	Nonanal	Saturated fatty Aldehyde		0.72		0.29	
40	9-Octadecenal	Aliphatic Aldehyde			2.43		
41	1-Decene	Alkene			1.46	1.05	
42	Dodecanamide	Fatty amide			0.75		
43	Butanone	Ketone				3.44	1.39
44	2-Octynoic acid	Fatty acid				0.34	
45	9-Hexadecenoic acid	Fatty acid				1.38	
46	Hexadecanal	Fatty Aldehyde				0.29	
47	Nonadecane	Fatty acid				0.30	
48	Benzene	Aromatic hydrocarbon				0.38	
49	2-Octen-1-ol					0.27	
50	3-Tetradecanone	Methyl Ketone				0.70	
51	N,N-dimethyl-pentadecylamine	Amine					61.21
52	Tetradecanal	Fatty Aldehyde					3.52
53	1,4-Cyclohexanediol						1.64
54	Diisooctyl phthalate	Ester					7.01
55	Hexadecanol	Fatty alcohol					1.50
56	Hexanoic acid	Carboxylic acid					2.30
57	Octadecenamide			2.39	36.02	5.06	

Table 2 Biomedical activities of terpenoid compound in kaffir lime cell suspension culture extracts.

No	Compound	Group	Peak area (%)					Biomedical activities
			Control	F5	F10	SC	IPP	
1	Alpha-Humulene	Sesquiterpene	0.32					Anticancer, antitumor, antimicrobial, antiinflammatory, analgesic and antioxidant [22]
2	Nerolidol	Sesquiterpene	0.89	0.31		1.55		antiparasite, antioxidant, antiinflammatory, and anticancer [23] antinociceptive, antimicrobial [24]
3	Farnesol	Sesquiterpene		1.54		2.03		antiinflammatory and anticancer [25]
4	Isopinocampheol	Monoterpene		0.39				Antifungal [26]
5	Eicosanol	Triterpene		2.78		5.11		Antitumor [27] and anticancer [28]
6	Beta-Pinene	Sesquiterpene				0.52		Antioxidant, antiinflammatory, antimicrobial, anticancer [29]
7	Beta-Carotene	Carotenoid				0.63		Antioxidant and anticancer [30]
8	Phytol	Diterpene				0.53		Antiantimicrobial [31], Antinociceptive, Antioxidant [32], antitumor, antiproliferation [33]
9	Squalene	Triterpene				1.60		Antioxidant, antitumor, antiproliferation [34]

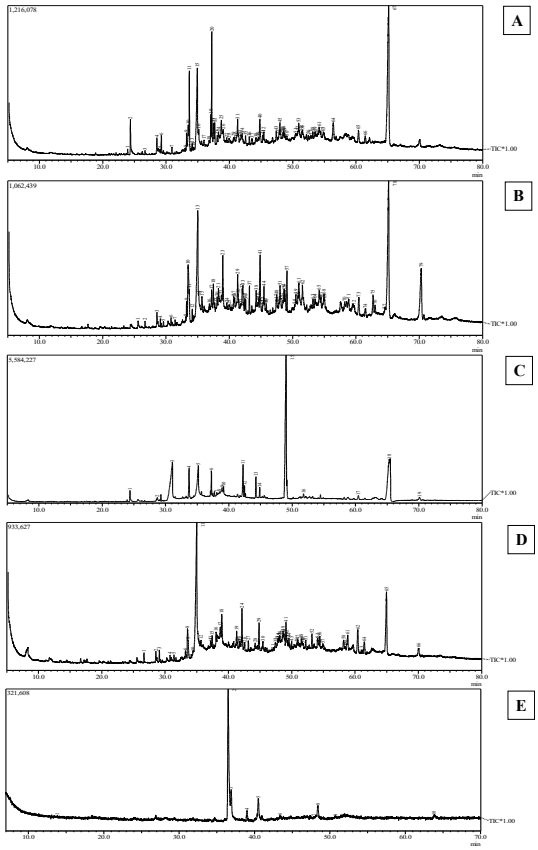

Fig. 5 GC-MS Total Ion Chromatogram of kaffir lime cell suspension extract: (A), Control; (B), F5; (C), F10; (D), SC; and (E), IPP.

They were classified as one flavonoid, nine terpenoids, and 56 of other groups. Bioactive compounds detected in all treatments were dominated by alkanes, fatty acids, and aldehydes. Some bioactive compounds (e.g. carboxylic acids, alkanes, alkenes, alcohols) found in kaffir lime cell suspension extracts were also found in *Phytolacca dodecandra* extracts. The compounds had antimicrobial and molluscicidal activities and could treat gonorrhea, rabies, malaria, ascariasis, jaundice, and eczema [35].

Interestingly, Alpha-humulene, one of the terpenoids, was only detected in the control treatment. Another member of terpenoids, nerolidol, was found in the control group and the F5 and SC treatments. Meanwhile, Farnesol and Eicosanol were found in F5 and SC treatments. Isopinocampheol was found only in the F5 treatment. Beta-Pinene, Beta-Carotene, Phytol, and Squalene were detected in the SC treatment (Table 2). These results showed that among the given treatments, F5 and SC treatments were able to induce more biosynthesis of bioactive compounds (particularly terpenoids) in the cell lines in comparison to the control group, whereas F10 and IPP treatments had no significant impact on the bioactive compound biosynthesis in the cell lines. Table 2 shows that SC produced more types of terpenoid compounds than the F5 treatment. Commercial technical-grade yeast is an *S. cerevisiae* yeast as well, but the treated kaffir lime cell produced different bioactive compounds. It could be explained that commercial technical-grade yeast consists of yeast (*S. cerevisiae*) and emulsifier (sorbitan monostearate E491), whereas pure-grade yeast contains 100% *S. cerevisiae* culture. The differences in these ingredients of elicitor might probably influence the production of bioactive compounds in kaffir lime cell suspension.

After the precursor treatment, the cells yielded fewer constituents than cells in the control group. In addition, there were also no terpenoid compounds identified in the cell extract. This might happen because 10 µg/ml of IPP was too high a concentration for kaffir lime, and the excessive IPP actually limited the terpenoid production. A previous study showed that a high concentration of IPP inhibited *E. coli* growth, and the reduction of cell viability was supposed to reduce the production of secondary metabolites [15]. Other studies stated that an excessive supply of IPP and DMAPP causeed impaired cell growth and accordingly

limited terpenoid production [13]. On the other hand, a low concentration of IPP precursor (0.01 mM) could enhance bilobalide and ginkgolide B production in the suspension cell culture of *Ginkgo biloba* [36].

Some biomedical activities were observed in the terpenoid compounds detected in this study. Regarding cancer studies, alpha-humulene could inhibit the cell proliferation of human hepatocellular carcinoma and induce cytotoxicity and apoptosis through the inhibition of Akt signaling [37]. Nerolidol was cytotoxic against the human colorectal cell line (Caco-2) and reduced the viability of Caco-2 cells [38]. Farnesol had cytotoxic effects against various neoplastic cell lines; it could significantly inhibit the growth of tumors in vivo by regulating multiple pathways in tumor cells, including the biosynthesis of PC, MEK/ERK, UPR, and JAK/STAT3; and the PI3K/Akt signaling cascade. Moreover, farnesol targeted different signal transduction pathways, making it a promising anticancer therapy [25]. Eicosanol could induce growth-suppression and apoptosis in Ca Ski cells, indicating that the compound could pay a role as an anticancer in cervical epidermoid carcinoma cells [28]. Thiazole-derived beta-pinene was used as an anticancer agent through mitochondrial-facilitated apoptosis in HeLa cells of cervical carcinoma, CT-26 colon cancer, and SMMC-7721 hepatocarcinoma. The compound dose-dependently inhibited cell proliferation by causing mitochondrial dysfunction signaling pathways; hence, stimulating cell cycle arrest [29]. Beta-carotene was able to induce apoptosis in MCF-7, MDA-MB-25, and MB-231 [39]. In addition, beta-carotene was able to reduce the activity of DLK1 (neural stem cell marker) in SK-N-BE neuroblastoma cells, indicating its ability to inhibit cancer cells [40]. Phytol had anti-angiogenic activity and induced apoptosis in A549 cells [33]. Squalene had antioxidant and antitumor activities and inhibited hyperproliferation in breast epithelial cells. In addition, squalene enhanced the immune system against tumors and protected breast cells against the accumulation of mutagenic lesions in DNA [34].

Overall, the present study succeeded in selecting terpenoid-producing cell lines of kaffir lime. The cytotoxicity of the kaffir lime cell line against cancer cells prompted further investigations. Moreover, a strategy to maintain the synthesis of terpenoids from the cell line needed to be elucidated; for example, subculture of the cell line for a set amount of time [12].

CONCLUSION

The number of living kaffir lime cells decreased as much as 37.1% to 40.4% after the F5, IPP, SC, and F10 treatments. The decrease of cell count indicated that cell line selection was successfully carried out. Bioactive compounds detected in all treatments were dominated by alkanes, fatty acids, and aldehydes. The F5 and SC treatments were able to induce more terpenoid biosynthesis in the cell lines.

Acknowledgements: This work was supported by Rekognisi Tugas Akhir (RTA) Program Universitas Gadjah Mada Batch I 2022, Grant Number 3550/UN1.P.III/Dit-Lit/PT.01.05/2022 (to WAST).

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