Growth and secondary metabolites production of Kaempferia galanga L. Callus under the influence of ultraviolet-B radiation

by Suprayogi Suprayogi

Submission date: 06-Mar-2023 03:42AM (UTC+0700)
Submission ID: 2029395540
File name: 14_Artikel_JMPS.pdf (583.92K)
Word count: 6119
Character count: 32489

Journal of Medicinal Plants Studies

www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2022; 10(1): 01-07

Received: 16-10-2021
Accepted: 02-12-2021

Shofiyani Anis
Department of Agrotechnology, Faculty of Agriculture and Fisheries, Universitas Muhammadiyah Purwokerto, Indonesia

Suwarto
Department of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Indonesia

Suprayogi
Department of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Indonesia

Yuniaty Alice
Department of Biology, Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia

Corresponding Author:
Shofiyani Anis
Department of Agrotechnology, Faculty of Agriculture and Fisheries, Universitas Muhammadiyah Purwokerto, Indonesia

Growth and secondary metabolites production of *Kaempferia galanga* L. Callus under the influence of ultraviolet-B radiation

Shofiyani Anis, Suwarto, Suprayogi and Yuniaty Alice

Abstract
Kaempferia galanga L. is one of the herbal plants that has been used for generations by the community because it has medicinal properties. The development of this plant continues to decline due to the conversion of land for food crops which results in the decreasing supply of quality herbal raw materials for the pharmaceutical and chemical industries. A promising alternative for developing medicinal plants capable of producing secondary metabolites without competing with land use for food crops is *in vitro* culture, especially callus culture. This study aimed to determine the effect of UV-B radiation on the growth, secondary metabolite production, and antioxidant capacity of *K. galanga* callus. The treatments included the intensity of ultraviolet-B radiation and radiation exposure time. The intensity of UV radiation with three levels, namely 70, 140, and 210 μW/cm^2, UV-B radiation exposure time was 2, 4, and 6 hours. Phytochemical parameters of UV-B radiation treatment had no significant effect on total phenol content. The combination of treatment intensity and duration of exposure affected the parameters of total flavonoid levels in *K. galanga* callus during the study, namely the combination of treatment intensity 140 μW/cm^2 and UV-B radiation exposure time of 2 hours, namely 53.94 ± 4.19 μg/g DW callus. At 2 hours of exposure to UV-B radiation, antioxidant activity showed the highest antioxidant ability, 49.51 ± 4.23%. PAL enzyme activity did not show any effect of UV-B elicitation on PAL enzyme activity to increase the content of phenolic compounds. GCMS test results showed that exposure to UV-B irradiation induces several compounds that have medicinal functions.

Keywords: *K. galanga* callus, UV-B elicitor, callus growth, secondary metabolites

Introduction
Kaempferia galanga L. is a valuable source of bioactive compounds. *K. galanga* is widely used as raw material for herbal medicines, phytopharmaca, food, and beverage flavoring, spices, and cosmetics (Narayanaswamy and Ismail, 2015) [1]. In the medical field, *K. galanga* is used as an anti-inflammatory and analgesic (Vittalrao *et al*., 2011) [2], treating headaches, toothache, rheumatism (Syahruddin, Dahlan, and Taslim, 2017) [3], antitumor, and cancer (Umar *et al*., 2011) [4], sedative activity (Huang, Yagura and Chen, 2008) [5], antimicrobial (Elya *et al*., 2016) [6], anthelmintic activity, antidiarrheal (Prites *et al*., 2014) [7], anti-larvacidal and medicinal mosquitoes (Liu *et al*., 2014) [8], because the rhizome extract of *K. galanga* contains essential oils with important phytoconstituents in the form of Ethyl-cinnamate and Ethyl p-methoxycinnamate which play a role in treatment (Shetu *et al*., 2018) [4].

The production of secondary metabolites of *K. galanga* plants for industrial needs is substantial. However, the availability of land for planting *K. galanga* is decreasing due to land use for commercial food crop cultivation. Coupled with the low interest of farmers in Indonesia to grow *K. galanga* plants, this has caused a decrease in the supply of raw materials for this herbal medicine for the pharmaceutical and cosmetic industries. Other problems related to the growth of *K. galanga* in the field are influenced by various environmental factors such as soil, nutrition, climate, and pest and disease attacks. One alternative to meet the needs of secondary metabolite products without competing with current land uses is *in vitro* culture technology (Kalpana and Anbazhagan, 2009) [10]. *In vitro*, culture techniques can be used for large-scale commercial propagation in the sustainable production of secondary metabolites in *K. galanga* (Sahoo *et al*., 2014) [11], sustainable and reliable (Vanisree *et al*., 2004) [12]. They can increase the production of secondary metabolites many times through callus and cell culture (Chattopadhyay *et al*., 2002) [13].

This success can be studied further by using an elicitor to determine its potential in producing

secondary metabolites in *K. galanga* to produce compounds with essential functions for the pharmaceutical and chemical industries under controlled conditions. One of the stress agents that can be used is a UV-B radiation elicitor. Ultraviolet radiation with a 400-200 nm wavelength has a biological impact that affects most living things on the earth's surface, including plants. However, only a tiny part of the radiation reaches the earth's surface. The response of plants to environmental changes, especially UV irradiation, is in the form of a protective mechanism or by activating repair mechanisms to overcome various types of stress. One standard protection strategy against potentially damaging UV radiation is synthesizing compounds that absorb UV radiation. Secondary metabolite compounds that absorb UV light are phenols, flavonoids, hydroxycinnamic esters, which accumulate in the vacuoles of epidermal cells, thereby preventing excessive penetration of the leaves (Frohnmeyer and Staiger 2003) [14], which have good effects as UV-B screening and UV-B defense functions in plants (Takshak and Agrawal, 2016) [15]. Defense metabolite products induced by UV-B elicitors such as phenols, flavonoids, and hydroxycinnamic esters have functions in many human treatments (Katerova *et al*., 2012) [16].

UV-B treatment negatively affected callus growth and cell size. UV-B radiation induces an increase in phenolic levels of flavan and lignin. The accumulation and localization of phenol compounds in tissues in response to UV-B radiation (intensity 0.74 W m^{-2} for up to 40 days) were studied in callus cultures of two *Camellia sinensis* L. strains, which varied in their biosynthetic capacity (Zagoskina *et al*., 2003) [17]. UV-B exposure to *P. quadrangularis* callus optimally increased the production of flavonoids 6 to 40 times that. UV-B treatment resulted in higher antioxidant activity compared to untreated callus (Antognoni *et al*., 2007) [18]. Elicitation by UV-B rays increase of camptothecin was observed in the *Camptotheca* cell culture, which has the potential to be an anti-tumor (Pi *et al*., 2010) [19]. UV-B treatment for the production of flavonoid compounds in *Jatropha curcas* L callus culture showed that irradiation of callus culture for seven days using two doses of UV-B induced vitexin, isovitexin, and apigenin synthesis. The three flavonoids increased 20 times more than the control in culture treated with a UV-B dose of 25.3 kJ / m^2.

Based on the explanation above, the purpose of this study was to determine the effect of UV-B radiation on callus growth, phenol production, flavonoids, the antioxidant capacity of secondary metabolites formed, and PAL enzyme activity callus. Tests with GCMS were carried out to determine the essential oil constituents in the ethanolic extract of *K. galanga* callus in this study.

Materials and Methods

Callus culture and conditions culture

K. galanga rhizome explants were obtained from the Research Institute for Spices and Medicines, Bogor. Indonesia. Callus was obtained from previous research through callus induction from *K. galanga* shoots with 2,4-D and BAP treatment. Callus in the fourth subculture was used in this study.

K. galanga callus was planted as much as 0.5 g of callus cells as inoculum into 30 ml of MS medium with the addition of 3 % sucrose, 8 g / L agar and 2,4-D 1.5 ppm. Callus was incubated in the environment where it grew under lighting a white fluorescent lamp with a light intensity of 1300 ± 50 lux at a temperature of 25 ± 2 °C.

Ultraviolet-B Radiation Treatment

The UV-B Exoterra TL 150 lamp with a power of 18 watts is used as a source of UV-B radiation at a distance of 20-30 cm from the callus (adjusted for the intensity to be used in the study). The exponential phase callus 4 weeks was used as the material treated with ultraviolet-B radiation according to the treatment. The intensity of radiation given to the callus was 70 $\mu W/cm^2$, 140 $\mu W/cm^2$, and 210 $\mu W/cm^2$. It was combined with an exposure time of 2, 4, and 6 hours and entire treatments received white light from fluorescent lamps to complete the light photoperiod for 16 hours with an incubation period of 2 weeks for all UV-B radiation treatments. The control treatment obtained a photoperiod of illumination for 16 hours. Harvesting was carried out at the end of the incubation period for whole treatments using UV-B radiation and control.

Growth Parameters

The growth parameters observed included: callus morphology, the color of the callus was observed by referring to the color score of the callus network using the "Royal Horticultural Society Color Charts edition V" RHSCF. Orgfree.com, callus fresh weight, callus dry weight, Growth Index (IP) used the following formula: Growth index = final fresh weight - initial fresh weight / initial fresh weight (Manaf *et al*., 2016) [20], observation of callus growth ratio using the following formula: Growth ratio = dry weight final - initial dry weight / initial dry weight (Lulu *et al*., 2015) [21].

Phytochemical Analysis of Callus

Sample Preparation

The sample tested was *K. galanga* rhizome callus aged six weeks after receiving UV-B radiation according to treatment. Sample preparation used a modified method (Subedi *et al*., 2014) [22], the ethanol extract of *K. galanga* callus obtained is stored at 4 °C, which will then be used for phytochemical tests such as measuring total phenol levels, total flavonoid levels, antioxidant tests with DPPH and GCMS tests.

Determination of total amount of phenol and flavonoid

The total amount of phenol in the callus ethanol extract was analyzed spectrophotometrically using the folin-ciocalteu method (Chandra *et al*., 2014) [23]. Gallic acid is used as the standard for determining the total amount of phenol content. The total phenol content was calculated as the gallic acid equivalent GAE / g dry weight of callus. Determination of flavonoid levels in callus ethanol extract was carried out using the aluminum chloride colorimetric method, and the total flavonoid content was calculated as the quercetin equivalent QE/ g dry weight of callus (Chandra *et al*., 2014) [23].

Analysis of antioxidant activity using the DPPH method

The percentage of DPPH radicle scavenging activities of callus ethanol extract was analyzed spectrophotometrically using the DPPH method (Sahoo *et al*., 2014) [11]. Ascorbic acid was used as a positive control, and the percentage of DPPH radicle scavenging activity was calculated by the following formulas: % inhibition = (Abs. Blank - Abs. Sample / Abs. Blank) x 100%.

PAL enzyme activity measurement

The measurement of PAL enzyme activity used a modified Zucker method (1969) (Rajabbeigi *et al*., 2013) [24]. The activity of the PAL enzyme was calculated as the equivalent of cinnamic acid formed. The protein concentration of the extract was determined according to the Lowry method (1951) (Randall and Lewis, no date) [25]. Enzyme activity is expressed as units/mg protein.

Test for essential oil compounds using GCMS

GC-MS analysis of callus essential oil was executed using Shimadzu QP-2010 gas chromatography combined with a mass spectrophotometer instrument (GC-MS) (Shimadzu Corporation, Japan). The essential oil components were identified by computerized matching of their peak mass spectra with data in the NIST 08, FFNSC 1.2, and Willey 8-Mass Spectral libraries of the GC-MS software system.

Statistical data analysis

Data were processed using the Costat version 6.400 computer software. Data that were normally distributed and homogeneous were analyzed using the Anova test. If the data does not meet the requirements, then the data analysis is carried out using the Kruskal-Wallis analysis. A further test was carried out using the Least Significance Different (LSD test) with a 95%.

Results and Discussion

Effect of UV-B on callus growth parameters

The effect of UV-B exposure intensity and time and its combination on growth parameters such as fresh weight, dry weight, growth index, growth ratio, callus morphology, and phytochemical analysis parameters of *K. galanga* callus are presented in Table 1. Treatment intensity and time UVB exposure did not affect all callus growth parameters. The morphology of the callus formed is a friable, with the color of the callus light brown to brown (Figure 1). There was no significant change in either the increase or decrease in callus growth rate with elicitation exposure to UV-B radiation given to all treatment combinations. This shows that UV-B radiation treatment at the various intensities and exposure time did not affect *K. galanga* callus's growth parameters during the study.

Effect of UVB on phytochemical analysis of callus *Kaempferia galanga* L.

Total Phenol and Flavonoids Callus

Testing of total phenol in callus with treatment intensity and exposure time of UV-B did not affect the total phenol content in callus. The combination of UV-B intensity treatment and UV-B exposure time significantly affected the total flavonoids formed in the callus. The combination of 210 $\mu W/cm^2$ UVB intensity treatment and 2 hours exposure time gave the highest total flavonoids, which was 53.94 ± 3.95 μg / g DW callus, which was not significantly different from the combination of 70 $\mu W/cm^2$ intensity treatment and 4 hours exposure time. 70 $\mu W/cm^2$ and an exposure time of 6 hours, an intensity of 210 $\mu W/cm^2$ and an exposure time of 2 hours and an intensity of 210 $\mu W/cm^2$ and an exposure time of 6 hours, respectively 49.55 ± 1.82; 46.86 ± 1.82; 44.46 ± 12.1 and 44.46 ± 5.55 μg / g DW callus.

DPPH callus antioxidant activity (%)

Callus culture showed antioxidant activity after UVB exposure time, where 2 hours of UV-B radiation exposure treatment showed the highest antioxidant activity, namely 49.51 ± 4.23%, which was not significantly different from the 4 hours and 6 hours exposure time, respectively. Each has an antioxidant activity of 39.78 ± 8.61% and 40.74 ± 8.35%, but significantly different from the control treatment, namely 37.71 ± 13.93%.

PAL Enzyme Activity

UV-B radiation treatment on *K. galanga* callus showed no effect on PAL enzyme activity. These results indicate that UV-B irradiation treatment did not change PAL enzymes' activity, which has a role in the formation of phenylpropanoid compounds in callus during the study. These results are directly proportional to the results of total phenol content in callus in this study. The data previously showed that UV-B irradiation did not affect the formation of total phenol content.

GCMS analysis of callus phytochemical components

The test results with GCMS on *K. galanga* callus treated with UVB showed that fatty acids dominated the components of the compounds formed. Ethyl p-methoxycinnamate (2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester), which is the most abundant compound in the rhizome of *K. galanga* was only formed in the lowest UV-B treatment, namely the combination treatment intensity of 70 $\mu W/cm^2$ and time of 2 hours by 5.1%. The radiation intensity treatment of 140 $\mu W/cm^2$ with an exposure time of 4 hours which was 52.27%, gave the highest hexadecanoic acid content as much as 52.27%, linoleic acid 16.78%, and oleic acid 8.1% (Table 2).

Discussion

Callus growth parameters, UV-B irradiation treatment had no significant effect because the environment and media for callus growth before treatment with UV-B radiation elicitation under uniform conditions allowed callus growth during the exponential phase to experience the same. In addition, the possibility of UV-B radiation treatment on the callus for two weeks in the exponential growth phase has not been able to influence callus growth significantly.

Callus morphology shows the formation of a friable textured and brownish color. Friable callus formation was due to using of a 2,4-D growth regulator to stimulate callus formation during the incubation period. The growth of *Chrysanthemum morifolium* callus derived from leaf explants with the addition of 2,4-D gave the callus a brownish yellow color (Setiawati *et al*., 2020) [26]. Another study showed that the addition of 2,4-D to the media resulted in yellowish callus on *Gynochthodes umbellata* callus and friable textured (Anjusha and Gangaprasad, 2017) [27]. The brownish color of the callus can also be due to the callus response to its environment, or the callus has entered a stationary phase, wherein secondary metabolite compounds begin to accumulate in the callus, such as phenolic compounds. These results contradict the results of research (Panchlaniya and Titov, 2018) [28], where continuous UV-B exposure for seven days with a duration of 2 hours each day for six weeks old callus has a positive effect on the increase in callus induction by 88% in combined *Glicine max*. with the addition of PGPR. Giving UV-B radiation was able to increase callus biomass, but in the period of increased exposure, it had a negative effect on inhibiting callus growth and decreasing cell survival (Ibañez *et al*., 2008; Manaf, Rabie and Abd El-Aal, 2016) [29, 20].

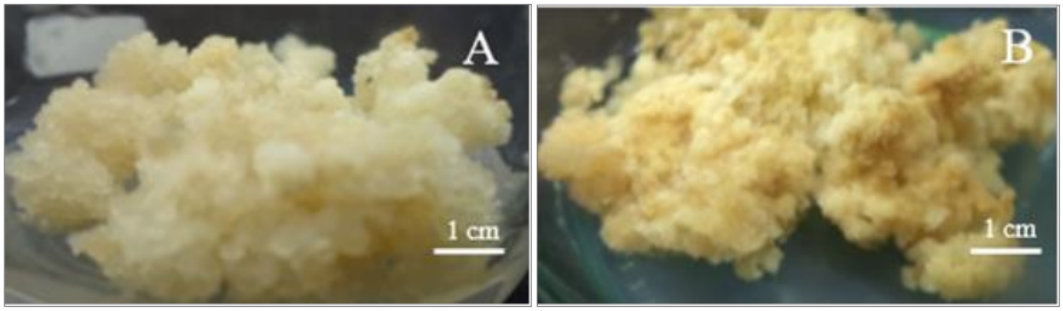

Fig 1: Morphology of callus without UV-B radiation (A) and callus with UV-B radiation (B)

Table 1: Effect of UV-B exposure intensity and time on callus growth parameters and phytochemical analysis of callus *Kaempferia galanga* L.

Treatment		Fresh Weight (g)	Dry Weight (g)	Growth Index	Growth Ratio	Total Phenol (µg/g DW)	Total Flavonoid (µg/g DW)	DPPH antioxidant activity (%)	PAL Enzyme Activity (unit/mg. protein)
UV-B intensity (I)	I0 (control)	4.98±0.49	0.19±0.02	9.97±0.98	9.75±0.82	561.34±156.78	39.97 ±7.35	37.71±13.93	4024.506±1046.23
	I1(70 μW/cm^2)	5.12±0.53	0.18±0.02	10.23±1.06	9.46±0.99	647.18±151.97	46.12 ±3.72	46.5 ±5.83	5791.85±2126.85
	I2(140 μW/cm^2)	4.99±0.47	0.18±0.02	10.00±0.94	9.35±1.19	639.43±267.46	44.79±8.14	41.95 ±10.56	4456.06±1161.12
	I3(210 μW/cm^2)	5.11±0.3	0.19±0.02	10.23±0.6	9.78±0.9	517.51±115.39	43.43±7.33	41.59 ±8.00	4340.48±1286.5
UV-B Exposure Time (T)	T0 (control)	4.98±0.49	0.19±0.02	9.97±0.98	9.75±0.82	561.36±156.78	39.97±7.35	37.71 ±13.93b	4024.506±1046.23
	T1 (2 hour)	5.06±0.53	0.17±0.02	10.12±1.07	9.13±1.11	561.75±136.81	46.79±8.49	49.51± 4.23a	4238.439±1092
	T2 (4 hour)	5.02±0.39	0.18±0.02	10.03±0.79	9.60±0.92	602.73±169.28	43.53±8.13	39.78 ±8.61ab	5261.507±2236.66
	T3 (6 hour)	5.15±0.38	0.19±0.02	10.31±0.76	9.86±0.96	639.63±262.78	44.03±4.64	40.74 ±8.35ab	5088.444±1591.61
Interactions (I x T)	I0T0 (control)	4.98±0.49	0.19±0.02	9.97±0.98	9.75±0.82	561.35±156.78	39.97±7.36b	37.71 ±13.93	4024.506±1046.23
	I1T1	5.22±0.73	0.17±0.02	10.44±1.47	9.01±1.13	564.40±57.76	41.96±2.11b	47.52 ±0.96	4729.790±277.87
	I1T2	4.80±0.21	0.18±0.02	9.60±0.41	9.25±0.88	643.30±108.20	49.55±1.82ab	45.99 ±6.18	6697.048±3351.18
	I1T3	5.33±0.45	0.19±0.01	10.65±0.90	10.11±0.74	733.82±236.16	46.86±1.82ab	45.99± 9.74	7716.049±2751.5
	I2T1	5.00±0.63	0.17±0.03	10.00±1.26	9.05±1.61	529.54±222.22	53.94±4.19a	52.42 ±3.95	3970.466±829.7
	I2T2	5.04±0.56	0.18±0.02	10.08±1.11	9.57±1.21	630.46±288.14	39.67±5.90b	33.95± 8.22	5543.169±1945.6
	I2T3	4.95±0.23	0.18±0.02	9.91±0.46	9.43±0.82	758.29±338.10	40.77±4.87b	39.49 ±9.64	4286.110±708.05
	I3T1	4.96±0.12	0.18±0.01	9.92±0.25	9.33±0.61	591.32±138.63	44.46±12.1ab	48.6± 5.94	4228.989±1131.03
	I3T2	5.20±0.30	0.19±0.01	10.40±0.6	9.97±0.64	534.43±96.33	41.37±5.33b	39.43 ±9.04	4781.313±1413.2
	I3T3	5.18±0.4	0.19±0.02	10.35±0.79	10.03±1.29	426.79±61.33	44.46±5.55ab	36.75 ±4.85	4327.115±1315.27

The numbers followed by the same letter are not significantly different in the 5% LSD test (value are the mean ± SD)

Table 2: The composition of the dominant chemical compound by GCMS testing on callus *Kaempferia galanga* L. in the treatment of UV-B radiation variations

No	R. Time	Compounds	I0T0	I1T1	I1T2	I1T3	I2T1	I2T2	I2T3	I3T1	I3T2	I3T3
			% Area									
1	2.52	2-hydroxymethy l-3-methyl-oxirane	-	-	-	-	-	-	-	-	-	8.98
2	2.57	2,3-eopxy-crotonsaeureisopropylamid	-	-	-	-	-	-	-	-	17.24	-
3	5.875	Trans (.beta.)-caryophyllene	-	-	-	-	-	-	-	-	-	7.96
4	2.621	S-(2-chloroethyl)-S-(2-hydroxyethyl)sulfide	-	-	-	-	-	-	-	-	-	10.03
5	6.655	Imidazolidine-1,3-dicarboxaldehyde, 4,5-diphenylamino-	-	-	-	-	-	-	-	5.97	-	-
6	6.981	Phenol, 2,4-bis(1,1-dimethylethyl)	4.42	4.7	9.05	5.31	-	-	-	-	-	-
7	8.402	4-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone	10.07	-	10.6	-	-	-	-	5.48	-	-
8	8.786	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	-	5.1	-	-	-	-	-	-	-	-
9	9.744	Methyl 13,14-dideutero-octadecanoate	-	9.19	-	-	-	-	-	-	-	-
10	9.963	Hexadecanoic acid (CAS)	-	-	32.92	38.84	37	52.27	38.1	21.6	21.18	21.1
11	10.136	Pentadecanoic acid, ethyl ester	-	-	19.54	-	-	-	-	-	-	-
12	10.146	Hexadecanoic acid, ethyl ester (CAS)	12.47	17.58	-	8.91	-	-	-	-	-	-
13	10.99	9,12-Octadecadienoic acid (Z,Z)- (CAS)	-	-	-	8.83	-	16.78	23.02	18.69	22.39	8.83
14	10.998	9-decenyl acetate	-	-	-	-	8.88	-	-	-	-	-
15	11.105	7(e)-dodecenyl acetate	-	-	-	-	13.27	-	-	-	-	-
16	11.11	Octadecanoic acid (CAS)	-	-	-	-	-	-	8.66	7.01	-	4.56
17	11.116	9-Octadecenoic acid (Z)- (CAS)	-	-	-	-	-	8.1	4.05	-	6.76	-
18	11.155	Undecanoic acid, 2,4,6-trimethyl-, methyl ester (CAS)	-	-	-	-	-	-	-	8.1	-	-
19	12.83	Nonane, 2-bromo-5-ethyl- (CAS)	18.67	-	-	-	-	-	-	-	-	-
20	12.835	14-.beta.-h-pregna	-	-	-	10.14	-	-	-	-	-	-
21	12.925	Ergost-25-ene-6,12-dione, 3,5-dihydroxy-, (3.beta.,5.alpha.)- (CAS)	15.1	-	-	-	-	-	-	-	-	-
22	12.949	Hexadecanoic acid, 2-(octadecyloxy)ethyl ester (CAS)	-	8.36	-	-	-	-	-	-	-	-
23	13.025	Sinigrin	-	10.02	-	-	-	-	-	-	-	-

UV-B radiation treatment on *K. galanga* callus did not affect some of the phytochemical parameters formed during elicitation. The treatment intensity and time of exposure to UV-B radiation did not affect the formation of phenolic compounds; however, UV-B radiation was affected by the formation of flavonoid compounds in callus *K. galanga*. Increased flavonoid production capacity in callus during the study as a photoprotective response due to UV-B radiation. UV-B elicitation in callus increases the production of ROS in tissues. The strategy carried out by the tissue is to use enzymatic and non-enzymatic scavengers such as phenolic flavonoids scavenge ROS and reduce UV-B penetration to protect photosynthetically acti tissue (Grace and Logan, 2000)[30]. The carbon framework synthesized by photosynthesis is dynamically used for growth (primary metabolism) or for defense (secondary metabolism) depending on the environmental conditions that occur (Caretto *et al*., 2015) [31]. In this case, the biochemical regulatory mechanisms that control carbon flux lead to the production of secondary metabolites in flavonoids for defense against UV-B radiation stress in the callus during the study.

The ability of callus antioxidant activity in this study was directly related to the total levels of flavonoids produced by callus due to UV-B radiation. Flavonoid antioxidants are secondary ROS scavenging systems in plants exposed to stress because flavonoids can inhibit the formation and reduce ROS after they are formed (Agati and Tattini, 2010) [31]. The ability of natural antioxidants that are formed is an effective photoprotector because of its ability to scavenge free radicals due to UV-B radiation and, at the same, time neutralize the reaction (Alvarez *et al*., 2019) [33], where B-ring substituted orthodoxy flavonoids are sub-strategy for class III peroxidase in reducing H_2O_2 enters epidermal cell vacuoles under severe light pressure and may be expanded to enter mesophyll cells (Yamasaki *et al*., 1997) [34].

The increase in secondary metabolite production comes from an increase in the enzymes' activities involved in phenolic pathways, including phenylalanine amonialiase and chalcone synthase enzymes; besides that, the activity of PEP (phosphoenolpyruvate) carboxylase enzyme must also increase because it is related to the relocation of sucrose production for the production of defense metabolites. The elicitation of UV-B radiation on PAL enzyme activity was not significant in this study is probably due to competition between proteins and phenylpropanoid synthesis in utilizing the amino acid phenylalanine precursor, wherein high growth conditions (exponential phase) protein synthesis for biomass needs can reduce the availability of phenylalanine for phenolic compound production (Caretto *et al*., 2015) [31].

Test results with GCMS on *K. galanga* callus treated with UVB showed that the components of the compounds formed were dominated by fatty acids such as hexadecanoic acid (CAS), linoleic acid (9,12-Octadecadienoic acid (Z,Z)-(CAS)), octadecanoic acid. acid (CAS), 9-Octadecenoic acid (Z)- (CAS) and hexadecanoic acid, ethyl ester (CAS). About 98.98% of essential oil constituents of the hexane extract of *K. galanga* rhizome have been isolated and identified, with only 1.11% of the components still unknown where the most abundant essential oil constituents include propanoic acid, pentadecane, ethyl p-methoxycinnamic (Huang, Yagura, and Chen, 2008) [5] Palmitic acid formed has potential antioxidant activity (Rajalakshmi *et al*., 2016) [35] and antineoplastic, antiandrogen, 5-Alpha reductase inhibitor, hypocholesterolemic agent (Ali *et al*., 2018) [36]. Linoleic acid is anti-inflammatory, hypocholesterolemic, cancer prevention, hepatoprotective, anti-arthritis, anti-corona (Ali *et al*., 2018) [36]. Oleic acid as anti-tumor, anti-inflammatory (Ali *et al*., 2018) [36]. In plants, fatty acids modulate various responses to various biotic and abiotic stresses, where levels of polyunsaturated fatty acids affect the fluidity of lipids in the chloroplast membrane and determine the ability of plants to adapt to stress, temperature, drought, salt, and heavy metal tolerance as well as response and defense caused by biotic factors (Kacrhroo, 2009) [37].

Research conducted by Pasillas *et al*. (2014) [38] with the elicitation treatment of jasmonic acid in suspension culture of *Catharamus roseus* cells showed the dominance of palmitic acid, linoleic acid, and α-linoleic acid in suspension culture. Phenol compounds, 2,4-bis(1,1-dimethyl ethyl), which are formed in several treatments, act as antioxidants because they can inhibit ROS activity (Teresa *et al*., 2014) [39], antibacterial and anti inflammatory activities (Ayinke *et al*., 2015) [40]. Ethyl p-methoxycinnamate (2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester), which is the most abundant compound in the rhizome of *K. galanga*, has anti-inflammatory and analgesic activity (Vittalrao. 2011)[2], headache treatment, toothache, rheumatism (Syahruddin *et al*. 2017) [3], antitumor and cancer (Umar *et al*. 2014, Ali *et al*. 2018) [41, 36], sedative activity (Huang *et al*. 2008) [5], anti-microbial (Tewtrakul *et al*. (2005) [42], Elya *et al*. 2016) [6], anthelmintic activity (Pritesh *et al*. al. 2014) [7], anti-larvicidal and mosquito repellent (Liu *et al*. 2014) [8], anti-fungal (Parjo *et al*. 2018) [43], antioxidant activity (Sahoo *et al*. 2014) [11], anti-thrombotic (Saputri and Avatara, 2018) [44], Hypopigmentary (Ko *et al*. 2014) [45].

Conclusion

This study reported the response of callus growth, the production of secondary metabolites of phenols and flavonoids, the antioxidant activity of the phenolic compounds produced, and the activity of PAL enzymes as a result of elicitation of UV-B radiation. The results showed that the growth parameters, including fresh callus weight, dry callus weight, growth ratio, and growth index of *K. galanga* callus, were not influenced by UV-B elicit. Likewise, for the phytochemical parameters where UV-B elicitation did not affect the total phenol content, but there was a change in the total flavonoid content in the *K. galanga* callus where the flavonoids produced had various antioxidant capacities. The total phenol content was related to PAL enzyme activity, which showed no effect of UV-B elicitation on PAL enzyme activity to increase the content of phenol compounds. Insufficient availability of nutrients and the growth phase (exponential phase) of callus when UV-B elicitation is one of the causes of reducing the impact of UV-B elicitation on the callus; besides that, it is possible that the intensity and time of UV-B exposure are still not able to induce significant changes to the growth parameters and phytochemical callus during the study. The GCMS test results show that exposure to UV-B radiation induces the formation of several compounds that have medicinal functions.

Acknowledgments

This research was funded by the Directorate of Research and Development, Ministry of Research and Technology / National Research and Innovation Agency, the Republic of Indonesia

Disclaimer: None

Conflict of Interest: None

Source of Funding: This study was supported by the RISTEK-DIKTI under grant Kept.631 / UN23.18 / PT.01.05 / 2020.

References

1. Narayanaswamy R, Ismail IS. Cosmetic potential of Southeast Asian herbs: an overview. Phytochem. Rev 2015;14:419-428.
2. Vittalrao AM, Shanbhag T, Meena Kumari K, Bairy KL, Shenoy S. Evaluation of anti-inflammatory and analgesic activities of alcoholic extract of *Kaempferia galanga* in rats. Indian J Physiol. Pharmacol 2011;55:13-24.
3. Syahruddin AN, Dahlan CK, Taslim NA. The Effects of *Kaempferia galanga* L. Extract on Pain, Stiffness and Functional Physic in Patient with Knee Osteoarthritis: Double Blind Randomized Clinical Trial. Int. J Sci. Healthc. Res 2017;2:37-43.
4. Umar MI *et al*. Phytochemistry and medicinal properties of *Kaempferia galanga* L. (Zingiberaceae) extracts 2011;5:1638-1647.
5. Huang L, Yagura T, Chen S. Sedative activity of hexane extract of *Kaempferia galanga* L. and its active compounds. J Ethnopharmacol. 2008;120:123-125.
6. Elya B, Kusuma IM, Jufri M, Handayani R. Antibacterial tests against acne *in vitro*, the physical stability and patch test using cream containing ethyl p-methoxycinnamate extracted from *Kaempferia galanga* L., Rhizoma. Res. J Med. Plant. 2016;10:426-434 .
7. Pritesh RD, Mahmuda N, Sheikh ZR, Ali MS. Study of Antidiarrhoeal Activity of Two. Int. J Pharm. Sci. Res. 2014;5:3864-3868 .
8. Zhang X, Liu C. Multifaceted Regulations of Gateway Enzyme Phenylalanine Ammonia-Lyase in the Biosynthesis of Phenylpropanoids. Mol. Plant. 2015;8:17-27 .
9. Shetu HJ, *et al*. Pharmacological importance of *Kaempferia galanga* (Zingiberaceae): A mini review. Int. J Res. Pharm. Pharm. Sci. 2018;3:32-39.
10. Kalpana M, Anbazhagan M. *In vitro* production of *Kaempferia galanga* (L.) - an endangered medicinal plant. J Phytol. 2009;1:56-61.
11. Sahoo S, Parida R, Singh S, Padhy RN, Nayak S. Evaluation of yield, quality and antioxidant activity of essential oil of *in vitro* propagated *Kaempferia galanga* Linn. J Acute Dis. 2014;3:124-130.
12. Vanisree M, *et al*. Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. 2004.
13. Chattopadhyay S, Farkya S, Srivastava AK, Bisaria VS. Bioprocess considerations for production of secondary metabolites by plant cell suspension cultures. Biotechnol. Bioprocess Eng. 2002;7:138-149.
14. Frohnmeyer H, Staiger D. Ultraviolet-B Radiation-Mediated Responses in Plants. Balancing Damage and Protection. Plant Physiol. 2003;133:1420-1428.
15. Takshak S, Agrawal SB. Ultraviolet-B irradiation: A potent elicitor of phenylpropanoid pathway compounds. J Sci. Res. 2016;60:79-96.
16. Katerova Z, Todorova D, Tasheva K, Sergiev I. Influence of ultraviolet radiation on plant secondary metabolite production. Genet Plant Physiol. 2012;2:113-144.
17. Zagoskina NV, Dubravina GA, Alyavina AK, Goncharuk EA. Effect of Ultraviolet (UV-B) Radiation on the Formation and Localization of Phenolic Compounds in Tea Plant Callus Cultures. Russ. J Plant Physiol. 2003;50:270-275.
18. Antognoni F, *et al*. Induction of flavonoid production by UV-B radiation in *Passiflora* quadrangularis callus cultures. Fitoterapia 2007;78:345-352.
19. Pi Y, *et al*. Examination of camptothecin and 10-hydroxycamptothecin in Camptotheca acuminata plant and cell culture, and the affected yields under several cell culture treatments. Biocell 2010;34:139-143.
20. Manaf HH, Rabie KAE, Abd El-Aal MS. Impact of UV-B radiation on some biochemical changes and growth parameters in Echinacea purpurea callus and suspension culture. Ann. Agric. Sci. 2016;61:207-216.
21. Lulu T, Park SY, Ibrahim R, Paek KY. Production of biomass and bioactive compounds from adventitious roots by optimization of culturing conditions of Eurycoma longifolia in balloon-type bubble bioreactor system. J Biosci. Bioeng. 2015;119:712-717.
22. Subedi L, *et al*. Antioxidant activity and phenol and flavonoid contents of eight medicinal plants from Western Nepal. J Tradit. Chinese Med. 2014;34:584-590.
23. Chandra S, *et al*. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. Evidence-based Complement. Altern. Med. 2014.
24. Rajabbeigi E, *et al*. Interaction of drought stress and UV-B radiation - Impact on biomass production and flavonoid metabolism in lettuce (*Lactuca sativa* L.). J Appl. Bot. Food Qual. 2013;86:190-197.
25. Randall RJ, Lewis A. The folin by Oliver.
26. Setiawati T, Ayalla A, Nurzaman M, Kusumaningtyas VA, Bari I. Analysis of Secondary Metabolites of Shoot, Callus Culture and Field Plant of Chrysanthemum morifolium Ramat. J ILMU DASAR. 2020;21:1.
27. Anjusha S, Gangaprasad A. Callus culture and *in vitro* production of anthraquinone in *Gynochthodes umbellata* (L.) Razafim. & B. Bremer (Rubiaceae). Ind. Crops Prod. 2017;95:608-614.
28. Panchlaniya R, Titov A. The study of effect on *Glycine max* (L.) Merrill tissue culture by plant growth regulators (PGRS) and Uv-B Supplementation. 16-25.
29. Ibañez S, Rosa M, Hilal M, González JA, Prado FE. Leaves of Citrus aurantifolia exhibit a different sensibility to solar UV-B radiation according to development stage in relation to photosynthetic pigments and UV-B absorbing compounds production. J Photochem. Photobiol. B Biol. 2008;90:163-169.
30. Grace SG, Logan BA. Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. Philos. Trans. R. Soc. B. Biol. Sci. 2000;355:1499-1510.
31. Caretto S, Linsalata V, Colella G, Mita G, Lattanzio V. Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. Int. J Mol. Sci. 2015;16:26378-26394.
32. Agati G, Tattini M. Multiple functional roles of flavonoids in photo protection. New Phytol. 2010;186:786-793.
33. Álvarez-Gómez F, Korbee N, Figueroa FL. Effects of UV Radiation on Photosynthesis, Antioxidant Capacity and the Accumulation of Bioactive Compounds in *Gracilariopsis longissima*, *Hydropuntia cornea* and *Halopithys incurva* (Rhodophyta). J Phycol. 2019;55:1258-1273.
34. Yamasaki H, Sakihama Y, Ikehara N. Flavonoid-peroxidase reaction as a detoxification mechanism of

plant cells against H2O2. Plant Physiol. 1997;115:1405-1412.
35. Rajalakshmi K, Mohan VR, Unit E, College VOC (Oleaceae) Stem by Gc-Ms Analysis. 2016;7:36-42.
36. Ali H, Yesmin R, Satter MA, Habib R, Yeasmin T. Antioxidant and antineoplastic activities of methanolic extract of *Kaempferia galanga* Linn. Rhizome against Ehrlich ascites carcinoma cells. J King Saud Univ. – Sci. 2018;30:386-392.
37. Kachroo A, Kachroo P. Fatty acid-derived signals in plant dfense. Annu. Rev. Phytopathol. 2009;47:153-176.
38. Goldhaber-Pasillas GD, Mustafa NR, Verpoorte R. Jasmonic acid effect on the fatty acid and terpenoid indole alkaloid accumulation in cell suspension cultures of *Catharanthus roseus*. Molecules. 2014;19:10242-10260.
39. María Teresa RC, Rosaura VG, Elda CM, Ernesto GP. The avocado defense compound phenol-2, 4-bis (1,1-dimethylethyl) is induced by arachidonic acid and acts via the inhibition of hydrogen peroxide production by pathogens. Physiol. Mol. Plant Pathol. 2014;87:32-41.
40. Ayinke A, *et al*. *In vitro* Evaluation of Membrane Stabilizing Potential of Selected Bryophyte Species. European J Med. Plants. 2015;6:181-190.
41. Umar MI, *et al*. Ethyl-p-methoxycinnamate isolated from *Kaempferia galanga* inhibits inflammation by suppressing interleukin- 1, tumor necrosis factor-α, and angiogenesis by blocking endothelial functions. Clinics. 2014;69:134-144.
42. Tewtrakul S, Yuenyongsawad S, Kummee S, Atsawajaruwan L. Chemical components and biological activities of volatile oil of. Sci. Technol. 2005;27:503-507.
43. Parjo NB, Mohamed Zulkifli R, Md Salleh M, Tencomnao T. Antidandruff potential of *Kaempferia galanga* ethanolic extracts for hair cream formulation. J Teknol. 2018;80:41-46.
44. Saputri FC, Avatara C. Xanthorrhiza Roxb. on Collagen-epinephrine Induced Thromboembolism in Mice. 2018;10:1149-1153.
45. Ko HJ, *et al*. Hypopigmentary effects of ethyl P-methoxycinnamate isolated from *Kaempferia galanga*. Phyther. Res. 2014;28:274-279.
46. Diriba Taye, Monenus Etefa. Review on improving nutritive value of forage by applying exogenous enzymes. Int J Vet Sci Anim Husbandry 2020;5(6):72-79.

Growth and secondary metabolites production of Kaempferia galanga L. Callus under the influence of ultraviolet-B radiation

ORIGINALITY REPORT

7%	5%	5%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	cyberleninka.org Internet Source	1%
2	Submitted to Higher Education Commission Pakistan Student Paper	1%
3	Ihtisham Umar. "Phytochemistry and medicinal properties of Kaempferia galanga L. (Zingiberaceae) extracts", African Journal of Pharmacy and Pharmacology, 2011 Publication	1%
4	download.atlantis-press.com Internet Source	1%
5	www.plantsjournal.com Internet Source	1%
6	Ajay Kumar. "Phytochemistry, pharmacological activities and uses of traditional medicinal plant Kaempferia galanga L. – An overview", Journal of Ethnopharmacology, 2020 Publication	1%

7	mdpi.com Internet Source	1 %
8	Germ, M.. "Influence of UV-B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins (Cucurbita pepo L.)", Plant Physiology et Biochemistry, 200505 Publication	1 %
9	Submitted to Universitas Negeri Surabaya The State University of Surabaya Student Paper	1 %
10	molecules.gnu-darwin.org Internet Source	1 %

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		