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Changes in Chemical Composition, Total Phenolics and Antioxidant Activity of Alpinia (*Alpinia zerumbet*) Leaves Exposed to UV

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Abstract. *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm is an important perennial ginger plant in the tropics with various uses as foods, dietary supplement, cosmetics, essential oil production and traditional medicines. In this study, essential oils, phenolic acids, fatty acids, kavains, total phenols and antioxidant activity in *A. zerumbet* plants exposed to UV-C were investigated. Number and weights of essential oils increased rapidly from 1 to 3 days of exposure. Major extend of essential oils was recorded in methyl cinnamate and followed by benzyl acetone. The correspondence to the accumulation of palmitic acid was 15 folds, in contrast to the strong reduction of stearic quantity was 10 folds at 3 days of treatment. Changes in chemical components and antioxidant capacity in *A. zerumbet* are differed reversely between the exposure of UV-C and heavy toxic chemicals. The exposure of UV-C is beneficial for the increasing of essential oil production in *A. zerumbet*.

Introduction

Higher plants are immobile therefore they cannot escape environmental stresses. Environmental and biotic stresses such as salts, UV, drought, wounding, and pathogen attacks are known to cause production of reactive oxygen species (ROS) causing them oxidative stress [1]. In general, energy-rich UV leads to the generation of free radicals which damage DNA, protein, membrane lipids and the photosynthetic machinery including chloroplasts and the degradation of photosynthetic pigments [2]. Plant exposure to high levels of UV radiation causes an oxidative stress [3] and results in leaf chlorosis and necrosis [4], which subsequently influences plant growth [5]. In particular, UV-C radiation induces in plant cells, apoptotic-like effects such as nuclear fragmentation and DNA laddering [6], a common feature of programmed cell death. It has also been reported that changes in antioxidant activities were associated with UV irradiation [7].

As plants are sessile organisms, they possess a number of strategies to cope with environmental stresses including UV radiation. Plants are known to synthesize and accumulate a spectrum of secondary metabolites, such as phenolics, saponins, cyanogenic glycosides, cyclic hydroxamic acids, sesquiterpenes, isoflavonoides, and sulfur-containing indole derivatives in response to physiological stimuli and stress [8]. Phenolic compounds are widely distributed in plants and contribute color and flavor to fruits and vegetables [9]. They additionally play a crucial role in ROS-associated plant resistance and defense against microbial infections. In addition, the other secondary metabolites, such as long chain fatty acids, phenolic acids, monoterpenes and kavains, are also have important roles in the defense system of higher plant [10]. Therefore, the production of these compounds may be closely relevant to the magnitude of environmental stresses.

Alpinia zerumbet (Pers.) B.L. Burtt. & R.M. Sm. (family Zingiberaceae) is an aromatic perennial ginger growing in tropical and subtropical regions and has been described to contain rich

essential oils, phenolics, and kavains which have been obtained and utilized for cosmetics and drugs [11,12]. In addition, traditional usage of this plant is reported to treat for various diseases and ailments and consumed as dietary supplement [11,13-19]. This herb obviously is well tolerated, and essential oils, phenolic acids, and kavains including dihydro-5,6-dehydrokavain (DDK) and 5,6-dehydrokavain (DK) as its main constituent are well characterized [11,17,18,20-23]. The use of *A. zerumbet* extracts and substituents derived from this plant as antihypertensive, antipsychotic, antidiabetic, antiplatelet, and antiulcer, antioxidant, and anti-HIV agents have been described [13-16,19].

In this study, we investigated the changes in contents of essential oils, fatty acids, DDK and DK, phenolic compounds and antioxidant activity in response to UV-C exposure in *A. zerumbet*.

Materials and Methods

Plant materials and treatment

A. zerumbet plants were cultivated by dividing the rhizomes into small pieces and planting in pots in a greenhouse at 25-30 °C. Eight-week-old and healthy plants were transferred to laboratory and placed in a UV chamber (1.69 width x 0.77 depth x 1.90 m height) equipped with two batches of 20 W germicidal UV-C lamp (G20T10 Sankyo Denki, Nagano, Japan), which were horizontally suspended on the top of the chamber. The plants were exposed to 34.5 kJm^{-2} UV-C dose with a flow density of 3.83 $mWcm^{-2}h^{-1}$, and similar treatments were repeated after 2 and 3 d. Untreated plants were the controls. After treatments, the plants were moved back to the greenhouse. Plant leaves in all trials were collected 24 h after irradiation for analysis. Experiments were performed triplicate in a completed randomized design. All chemicals were purchased from Wako Co., Japan.

Extraction of essential oils

One hundred grams of non-treated and UV-treated fresh leaves of *A. zerumbet* were subjected to steam-distillation for 4 h. The distillates were extracted with diethyl ether. The solvent was removed and essential oils were dissolved in diethyl ether and subjected to GC-MS analysis.

Preparation of the extracts

Leaves of non-treated and UV-treated plants were collected and placed in boiling water for 20 min. After cooling at room temperature, water extracts were filtered and successively extracted with chloroform and ethyl acetate. Obtained extracts were filtered, dried under vacuum and then dissolved in corresponding solvents for further analysis.

Amount of total phenolic compounds

Total phenols in ethanol extract with similar extracting procedure as described above was prepared and determined according to the Folin-Ciocalteu procedure [24]. An aliquot of 1.0 ml Folin-Ciocalteu's reagent and 800 µl 7.5% (w/v) Na_2CO_3 were added to 200 µl of plant extract dissolved in ethanol. After shaking, the mixture was incubated at room temperature for 30 min. Absorption was measured at 765 nm using a Shimadzu UV-160A spectrometer. Total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram extract.

Antioxidant assay using DPPH radical scavenging system

The radical scavenging activity was evaluated following a method described previously [25]. An aliquot of 2 ml plant extract dissolved in ethanol were mixed with 1.0 ml 500 µM DPPH ethanol solution and 2 ml 100 mM acetate buffer (pH 5.5). After shaking, the mixture was incubated at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm. Radical scavenging activity was expressed as percent inhibition and was calculated using the formula of Son and Lewis [26]:

$$\% \text{ radical scavenging activity} = (A_{control} - A\ t_{est}) / (A_{control}) \times 100$$

$A_{control}$ is the absorbance of the control (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus plant extract). The data was expressed in EC_{50} (effective concentration), which refers to the concentration which induces a response halfway between the baseline and maximum after a specified exposure time.

GC-MS analysis

A 1 µl aliquot of acetone solution of chloroform and ethyl acetate extract of non-treated and UV-treated plants was injected into the GC-MS (QP-2010, Shimadzu Co., Japan). The DB-5MS column was 30 m in length, 0.25 mm i.d. and 0.25 µm in thickness (Agilent Technologies, J&W Scientific Products, USA). The carrier gas was helium. The GC oven temperature program was 50 °C (6 min), 50-280 °C (5 °C min^{-1}), 280 °C (5 min). The injector and detector temperatures were set at 250 and 280 °C, respectively. The control of the GC-MS system and the data peak processing were carried out by means of the Shimadzu's GC-MS solution Software, version 2.4.

For essential oil analysis, an aliquot of 1 µl oil dissolved in diethyl ether was injected into the GC-MS using the same column described above. The carrier gas was helium and the GC oven temperature program was: 40 °C (5 min), 40-280 °C (6 °C min^{-1}), 280 °C (5 min). The injector and detector temperatures were set at 250 °C and 280 °C, respectively. The essential oil components were identified by comparing their retention times and mass fragmentation patterns with those of standards and MS library. Quantitative determinations were carried out based on peak area measurements. Similar identification and quantification of two fatty acids including palmitic acid and stearic acid, were also carried out.

Quantification by HPLC

DDK and DK were isolated from Alpinia leaves according to the method described previously [22] and were purified to be used as a standard. Benzoic acid, ferulic acid, and *trans-p*-coumaric acid were purchased from Wako Co., Japan. These compounds were measured at 280 nm using a Shimadzu HPLC (SCL-10 A vp. Shimadzu Co., Japan). Separations were achieved on a RP-18 ZORBAX ODS column (Agilent Technologies, USA) (25 cm x 0.46 cm i.d.: 5 µm particle size). A gradient elution was performed with solvent A (water:acetic acid, 99:1, v/v) and B (methanol:acetonitrile:acetic acid, 95:4:1, v/v/v) as follows: 0-2 min, 5% B; 2-10 min, 5-25% B; 10-20 min, 25-40% B; 20-30 min, 40-50% B; 30-40 min, 50-100% B; 40-45 min, 100% B; 45-45 min, 100-5% B. The flow rate was 1.0 ml/min and the injection volume was 5 µl. Identification of the compounds was carried out by comparing their retention times to those of standards.

Statistical analysis

All treatments were arranged in a completely randomized design with three replicates. Data were analyzed by SAS computer software version 6.12, SAS Institute [27] using ANOVA with the least significant difference (LSD) at the 0.05 probability level.

Results and Discussion

Chemical composition of essential oils

The essential oils of non-treated and UV-treated fresh leaves of *A. zerumbet* were obtained as yellow oils with aromatic spicy odor. The number of chemicals in essential oil extract increased rapidly from 1d, 2d, and 3 d (38, 52, and 63 compounds, respectively) as compared with that of the control (27 compounds) (Table 1). By comparing the percentage peak area, there are 34 compounds with amounts that were increased consecutively from 1-3 d of treatment, whereas that of 18 compounds was increased after 1 or 2 d, but decreased by 3 d. In contrary, the quantities of 11 constituents were reduced successively from 1-3 d (Table 1).

Among the 4 individual essential oils linalool, 4-tepieol, methyl cinnamate, and benzyl acetone, the weights of linalool and 4-tepieol were promoted at 1-2 d, but were strongly reduced at the 3 d point. However, in the case of benzyl acetone, its quantity was first reduced after 1 d, but it

was consecutively promoted at 2 and 3 d. For methyl cinnamate, the trace of this compound was not detected in both the control and 1 d of UV-C exposure, but it was found by 2 and 3 d, and the weight was simultaneously increased rapidly from 3.29 to 15.50 mg/g, respectively (Table 4).

Findings from Table 1 reveal that the influence by UV-C exposure to essential oils of *A. zerumbet* was opposite to treatment of copper sulphate [17]. In this study, the amount of essential oils was increased, but in the copper sulphate it was decreased. Moreover, the number of essential oils was reduced from 62 to 59 compounds by copper sulfate treatment [17], whereas in contrast it was extended rapidly from 27 to 38, 52, and 63 compounds after 1, 2, and 3 d of UV-C exposure. Among the detected 62 essential oils, the weights of 40 compounds were reduced, in contrast to that of 20 substances, which was increased in weight [17].

Table 1. Chemicals detected in ethyl acetate extract of Alpinia leaves treated by UV

No	Chemicals	Retention time	RI	Peak area (%)			
				Control	1 day	2 day	3 day
1	Benzaldehyde	12.1	909	0.10	0.05	0.04	0.05
2	*β*-Myrcene	13.0	987	0.03	0.03	0.10	0.01
3	3-Hexenoic acid	13.5	1002		-	0.05	0.01
4	*o*-Cymene	14.1	1022	0.70	0.07	0.09	0.30
5	Cineole	14.4	1030	2.43	0.09	1.11	4.33
6	Hypnone	15.4	1064	-	-	0.02	0.04
7	3-Heptenoic acid	16.0	1084		0.08	0.02	0.04
8	Linaloloxide	16.1	1085	0.08	0.09	0.02	0.04
9	Linalool	16.5	1100	1.31	1.42	0.72	1.58
10	Phenyethyl alcohol	16.9	1115	-	-	0.33	0.22
11	*trans*-*p*-Methal-2,8-dienol	17.1	1123	-	0.01	0.14	0.06
12	4-Isopropyl-1-methyl-2-cyclohexen-1-ol	17.2	1126	-	0.14	0.20	0.20
13	(-)-*cis*-Sabinol	17.6	1139		0.19	0.11	0.26
14	±-Camphor	17.8	1148	1.49	0.90	2.12	5.09
15	Camphene hydrate	18.0	1157	-	-	-	+
16	3-Isopropenyl-1,2-dimethylcyclo-pentanol	18.1	1158	-	0.24	0.23	0.34
17	Pinocarvone	18.3	1164	-	-	-	+
18	*p*-menth-1-en-8-ol	18.5	1172	-	-	-	+
19	Borneol	18.6	1176	-	-	1.33	1.86
20	4-Terpinenol	18.8	1182	0.62	0.83	1.92	2.28
21	Cryptone	19.0	1188	3.96	4.64	4.53	3.99
22	*α*-Terpineol	19.2	1197	0.71	2.01	2.52	2.42
23	Sabinyl acetate	19.4	1205	-	-	-	+
24	2-Pinen-4-one	19.6	1209	0.14	0.14	0.20	0.24
25	*cis*-Carveol	19.8	1221	-	-	-	+
26	1,4-Cineole	20.2	1236		0.33	0.23	0.13
27	Cuminal	20.4	1243	0.89	1.27	1.53	0.43
28	Benzylacetone	20.4	1245		0.10	0.16	0.58
29	Thymoquinone	20.5	1250	-	-	-	+
30	(2E)-2-Ethylidene-1,7,7-trimethylbi-cyclo [2.2.1]heptane	20.6	1251	0.25	0.26	0.21	0.14
31	3-Carvomenthenone	20.7	1255	0.45	0.51	0.43	0.13
32	Myrtenol	21.3	1277	-	-	0.23	0.06
33	Phellandral	21.3	1279	0.33	0.35	0.49	0.19

34	1,3,3-Trimethyl-2-vinyl-1-cyclo-hexene	21.6	1289	-	-	0.02	0.07
35	Carvacrol	21.8	1298	-	-	-	+
36	4,4,7a-Trimethyl-2,4,5,6,7,7a-hexa-hydro-1H-inden-1-one	21.8	1299	-	-	1.26	0.74
37	Methyl cinnamate	21.9	1301	-	-	0.55	0.19
38	Cuminol	22.0	1302	-	0.56	0.55	0.08
39	*p*-tert-Butylbenzylalcohol	22.2	1305	-	1.06	0.42	0.11
40	Acetylcyclopentanone	22.5	1309	-	-	2.93	1.99
41	Hydrocinamic acid	22.6	1311	0.26	4.62	4.78	0.19
42	Benzylmalonic acid	22.7	1312	-	-	0.70	0.08
43	3-Methyl-2-(3-methylpentyl)-3-buten-1-ol	23.1	1318	-	-	0.18	0.22
44	Isopinocampheol	23.2	1320	-	-	-	+
45	Geranyl propionate	23.7	1327	-	-	-	+
46	Barosma camphor	24.7	1339	-	-	1.37	0.53
47	Caryophyllene	24.8	1344	0.46	2.03	2.30	1.35
48	5-Ketoborneol	25.1	1347	-	-	1.18	0.06
49	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-, trans-	25.3	1351	-	0.81	1.93	0.38
50	*α*- Caryophyllene	25.7	1356	-	-	-	+
51	Butylated hydroxytoluene	26.6	1369	1.72	1.73	3.89	1.64
52	τ-Muurolene	26.8	1374	0.17	0.16	2.50	0.66
53	Carotol	26.9	1374	-	0.44	0.25	0.98
54	Hedycaryol	27.7	1386	0.09	0.41	0.13	0.54
55	±-*trans*-Nerolidol	27.8	1388	0.52	2.57	1.51	3.25
56	Epoxycaryophyllene	28.4	1398	1.48	4.86	0.28	4.51
57	Ledol	28.6	1399	-	-	-	0.19
58	Juniper camphor	29.5	1440	0.22	0.51	0.56	0.88
50	*α*-Cadinol	29.9	1462	-	-	1.91	3.08
60	Nonahexacontanoic acid	32.5	1596	0.02	0.20	0.23	0.13
61	Palmitic acid	35.4	1158	0.07	0.12	0.68	0.59
62	DDK	38.2	1733	8.93	24.3	23.5	10.7
63	DK	41.6	2153	1.14	1.42	1.28	0.03

-: not detected; +: detected, but the peak % < 0.01%; RI: Retention Index relative to *n*-alkanes on the DB-5 column.

The quantity of methyl cinnamate and benzyl acetone was strongly promoted, whereas that of linalool and 4-tepieol was reduced (Table 1). But by copper sulphate exposure, amount of methyl cinammate was reduced and the trace of benzyl acetone was not found. Also, a quantity of both linalool and 4-tepieol was increased [17]. It is proposed that the defense mechanism of *A. zerumbet* differs in contrast between exposures to UV-C and copper sulphate.

Chemical composition in chloroform and ethyl acetate extracts

Similar to essential oils, the number of chemicals detected in the chloroform (9, 10, and 11 compounds) and ethyl acetate (19, 19, and 26 compounds) extracts increased consecutively by 1, 2, and 3 d of UV-C exposure, respectively (Tables 2 and 3). Comparing the percentage peak area with that of the controls, there were 5 and 11 substances in chloroform and ethyl acetate layers which showed greater quantities in UV-C exposure (Tables 2 and 3).

Table 2. Chemicals detected in chloroform extract of Alpinia leaves treated by UV

No	Chemical name	Retention time	Peak area (%)			
			Control	1 day	2 day	3 day
1	3-Hexenoic acid	3.66	-	-	-	0.21
2	Benzyl Alcohol	4.76	-	1.98	0.02	0.66
3	Benzyl acetone	8.74	0.18	0.16	0.49	0.35
4	Methyl cinnamate	11.73	-	-	1.71	4.24
5	1.2-Isopropyl-2,5-dimethylcyclo-hexanone	12.6	1.53	1.65	0.99	2.02
6	1.3-(Hydroxymethyl)-6-isopropyl-2-cyclohexen-1-one	14.9	1.77	1.34	0.67	0.87
7	Phenanthrin	18.28	4.65	1.10	0.58	2.06
8	Dihydro-5,6-dehydrokavain (DDK)	24.23	13.4	8.05	15.61	8.94
9	5,6-Dehydrokavain (DK)	27.21	15.47	10.53	13.99	1.45
10	Chalcone, 2',4'-dihydroxy-5'-methoxy	31.15	0.25	1.29	1.69	0.59
11	*β*-Sitosterol	31.43	-	-	-	0.41

-: not detect.

Table 3. Chemicals detected in ethyl acetate extract of Alpinia leaves treated by UV

No	Chemical name	Retention time	Peak area (%)			
			Control	1 day	2 day	3 day
1	*trans*-3-hexenoic acid	3.79	-	-	-	+
2	3-Hexenoic acid	3.99	-	-	-	+
3	Glutaconic anhydride	4.12	8.67	3.89	7.56	3.66
4	Benzoic acid	6.93	3.12	0.66	0.60	1.69
5	Coumaran	8.16	2.05	2.11	2.96	2.22
6	Phenylmalonic acid	8.81	-	0.43	0.10	2.39
7	Cumic alcohol	9.91	1.51	0.94	1.16	1.65
8	*p*-Vinylguiaiacol	10.25	-	-	-	+
9	4-Hydroxy-2-methylacetophenone	10.26	4.80	3.58	3.60	0.99
10	Benzenepropanoic acid	10.52	0.51	1.43	0.52	2.61
11	Methyltriacetolactone	11.00	-	0.39	1.10	0.44
12	Cinnamic acid	12.53	-	-	-	1.06
13	*m*-Ethoxybenzoic acid	14.10	-	2.53	0.85	0.50
14	*p*-Salicylic acid	14.23	-	-	-	2.79
15	Vanillic acid	15.00	1.09	2.40	2.42	3.00
16	2-Hydroxymethylene-6-isopropyl-3-methylcyclohexanone	15.4	-	-	-	+
17	*m*-Coumaric acid	17.65	-	1.13	0.95	0.88
18	3-Hydroxy-4-methoxycinnamic acid	18.46	1.81	1.57	1.73	0.80
19	*trans-p*-Coumaric acid	19.05	2.40	3.57	3.07	3.75
20	Methyl ester-ferulic acid	19.81	0.55	0.55	0.38	1.19
21	Ferulic acid	20.32	15.21	15.76	19.09	7.27
22	Palmitic acid	21.61	-	1.37	1.56	2.19
23	Dihydro-5,6-dehydrokavain (DDK)	24.23	1.95	2.34	1.23	0.67
24	Stearic acid	24.44	0.77	3.18	0.42	0.42
25	5,6-dehydrokavain (DK)	27.21	0.98	2.18	2.20	0.59
26	Chondrillasterol	29.1	-	-	-	+

-: not detected;

+: detected, but the peak % < 0.01%.

Composition of fatty acids, kavains, and phenolic acids

The amount of palmitic acid increased 5 times by 1 and 2 d, and 15- fold by 3 d of UV-C exposure, whereas in contrast, that of stearic acid was reduced 2- and 3-fold by 1 and 2 d, respectively, and 10 times by 3 d (Table 4). Structurally, palmitic acid and stearic acid are long-chain saturated fatty acids with 16 and 18 carbons, respectively. In animals, under certain conditions, stearic acid can be converted into palmitic acid [28]. It is therefore hypothesized that under UV exposure, the stearic acid in *A. zerumbet* was transformed to paltimic acid to explain why palmitic acid rapidly increased whereas stearic acid was strongly decreased (Table 4).

Table 4. Content of major chemicals in Alpinia leaves treated by UV

Treatments	Essential oils (mg/g fresh leaves)			
	Linalool	4-Tepieol	Methyl cinnamate	Benzyl acetone
Control	0.17 ± 0.05b	0.16 ± 0.05b	0.00 ± 0.00c	0.94 ± 0.20c
1 day	0.25 ± 0.10a	0.18 ± 0.04b	0.00 ± 0.00c	0.74 ± 0.30c
2 day	0.19 ± 0.05ab	0.80 ± 0.02a	3.29 ± 0.20b	1.86 ± 0.50b
3 day	0.04 ± 0.01c	0.11 ± 0.05c	15.50 ± 2.30a	2.69 ± 0.70a
	Fatty acids (µg/g fresh leaves)			
	Palmitic acid		Stearic acid	
Control	2.00 ± 0.05c		60.00 ± 8.00a	
1 day	10.00 ± 2.50b		30.00 ± 12.00b	
2 day	10.00 ± 3.00b		20.00 ± 6.00b	
3 day	30.00 ± 4.50a		6.00 ± 1.15c	
	Kavain (mg/g fresh leaves)			
	Dihydro-5,6-dehydrokavain (DDK)		5,6-Dehydrokavain (DK)	
Control	4.94 ± 0.55a		1.39 ± 0.04a	
1 day	3.17 ± 1.22c		0.58 ± 0.15b	
2 day	3.88 ± 1.65bc		0.62 ± 0.24b	
3 day	3.38 ± 0.93bc		0.31 ± 0.13c	
	Phenolic acids (mg/g fresh leaves)			
	Benzoic acid	*trans-p*-coumaric acid	Ferulic acid	
Control	0.03 ± 0.01a	0.18 ± 0.05c	0.56 ± 0.15b	
1 day	0.01 ± 0.02b	0.35 ± 0.10b	0.76 ± 0.12a	
2 day	0.04 ± 0.01a	0.43 ± 0.08b	0.43 ± 0.07b	
3 day	0.03 ± 0.02b	0.51 ± 0.25a	0.49 ± 0.16b	

Each value represents the mean of three replicates ± SE (standard error). Means within columns with the same letter are not significantly different at $P < 0.05$.

For kavains, the quantity of DDK and DK was decreased interminably after 1-3 d of exposure (Table 4). This is the reserve of the treatment by copper sulphate which caused significant increase of both DDK and DK [17]. In this study, the quantity of *trans-p*-coumaric acid was markedly increased, whereas the amount of benzoic acid and ferulic acid was negligible (Table 4). Under the exposure to copper sulphate, the amounts of phenolics as well as vanillin and cinnamic acid were significantly increased [17]. It is also in contrast to the treatment of UV, except that of *trans-p*-coumaric acid. This phenolic acid is a derivative of cinnamic acid, suggesting that under either heavy chemical or UV exposures, cinnamic acid and its derivatives are important chemicals for the defense system of *A. zerumbet*.

Total phenol content and antioxidant activity measured by DPPH radical scavenging method

The smaller value of EC_{50} indicates stronger activity of DPPH radical scavenging capacity. It is observed that the antioxidant capacity was reduced markedly from 2-3 d after UV-C exposure (Table 5). After 1 day, the difference in DPPH radical scavenging capacity as compared to the controls was negligible, but it was strongly decreased by 2-3 d. The reduction of antioxidant

capacity may be attributed to the reduction of total phenols and some phenolic acids (Tables 4 and 5).

Table 5. Antioxidant activity and total phenols of Alpinia leaves treated by UV

Treatment	Antioxidant activity (EC_{50}) (µg/ml)	Total phenols (gallic acid equivalent mg/g extract)
Control	57.51 ± 6.55c	108.27 ± 9.87a
1 day	58.90 ± 5.70c	97.78 ± 5.92b
2 day	73.66 ± 9.51b	82.25 ± 11.92b
3 day	97.36 ± 12.24a	71.08 ± 4.74bc

Each value represents the mean of three replicates ± SE (standard error). Means within columns with the same letter are not significantly different at $P < 0.05$.

The magnitude of antioxidant capacity reduced by UV-C exposure was 41.2% (Table 4), whereas by treatment of copper sulphate it was increased by 12.4-21.0% [17]. Total phenols of the UV-C exposure by 3 d were lowered by 33.7% (Table 5), similarly, in treatment of copper sulphate, the total phenols in leaves of *A. zerumbet* were significantly decreased [17]. The stronger DPPH radical scavenging activity and greater total phenols of *A. zerumbet* treated by copper sulphate was corresponded to the increase of phenolic compounds [17]. The changes in amounts of DDK and DK may not influence antioxidant activity of *A.zerumbet* as the antioxidant capacity of DDK and DK is in rather low magnitudes [23].

The illumination of UV-C increased levels of flavonoids, phenolics, and anthocyanins, and antioxidant activity in blueberries (*Vaccinium corymbosum* L.) was reported [29]. Similarly, it was also noted that the treatment of UV-C reduced the severity of decay during storage of strawberry fruit (*Fragaria xananassava*) by promoting the antioxidant capacity and enzyme activities, and contents of phenolics and anthocyanins [30]. The exposure of UV-C resulted in an increase of total phenolics and flavonoids, and antioxidant capacity in leaves of lettuce (*Lactuca sativa* L.) [31]. In general, the use of UV-C is beneficial in treatments against bacteria, fungi, and diseases, occurring during storage of crops and foods. For instance, the UV-C radiation and fluorescent light was useful to control postharvest soft rot in potato seed tubers [32].

Conclusions

In this study, the exposure of UV-C on *A. zerumbet* resulted in rapid increase of number and quantity of essential oils, suggesting that the use of UV-C is beneficial for increasing essential oil production in *A. zerumbet*. Among them, methyl cinnamate and benzyl actetone increased the most. The exposure of copper sulphate on this plant [17] obtained a reverse result as compared to that of UV-C irradiation. In addition, the numbers and amounts of essential oils were reported to have been reduced, whereas the quantities of DDK and DK, and phenolics were significantly increased. Findings of this study show that the use of UV exposure is beneficial to increase production of essential oils in *A. zerumbet*, thus the efficacy from use of this plant as foods, dietary supplement, and cosmetics are therefore extended.

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