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Screening of Phytochemical Compounds of *Gigantochloa scortechinii* Bamboo Rhizome

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Absract

Bamboo leaves, culms, and roots are the most common bamboo part being studied regarding phytochemical functional properties. Many studies on various rhizomatous plant species, however, found potent functional properties of their rhizome part. This led an incline to explore the potential of bamboo rhizome and hence to promote its utilization. The aim of this study was to assess the variation of phytochemical compounds in the ethanolic extracts of *Gigantochloa scortechinii* bamboo rhizome. Destructive sampling was conducted using selective random sampling method on four consecutive rhizomes from healthy clumps at two natural forests and one planted forest. Homogenized sample were extracted using solvent extraction (70% ethanol) method. The extracts were analyzed using Gas Chromatography-Mass Spectrometry to identify the composition and concentration of phytochemical compounds. Screening process is important to get a clear figure of the compounds present in a plant and also important to assess whether presence of harmful compound. Results indicate that a total of 56 compounds were identified and distinguished between study site and rhizome age. The variation of its composition and concentration are suggested to be more affected by age-related factor with regard to variation of compound composition and concentration. Results also indicate that *G. scortechinii* rhizome contain various phytochemical compounds with potential as a plant of phyto-pharmaceutical importance.

Keywords: Bamboo; age-related; GC-MS; Solvent extraction, Phytochemical

INTRODUCTION

High value added products from bamboo particularly in term of pharmaceutical, cosmeceutical, and functional food have been produced in bamboo producer countries [1,2]. Those products produced since ancient version and recently, scientist come out with scientific evident regarding application and functional properties [1,3-7]. Recognizing the adverse effect of various synthetic health-related products (such as butylated hydroxytoluene (BHT) and paraben of others), finding for a new range of novel health effects of phytochemical compounds among natural products such as antioxidant of bamboo leaves are increased in demand and considered with human health [8-13].

Phytochemical compounds are biologically active non-nutrients derived from intermediary secondary metabolites of growing xylem and phloem. There are synthesized through biosynthetic pathway, i.e. a) nucleoside diphosphate sugar pathway, b) shikimate-cinnamate pathway, c) acetate-cinnamate pathway, d) mevalonate pathway and e) mixed pathway of c and d pathway [12,14]. Regarding a large number of phytochemical compounds and the range of potential functional properties, various and sophisticated experimental approaches must be occupied to increase the understanding of its complex biology. Therefore, the identification of phytochemical compounds and determination of their concentrations are necessary before measuring the functional properties, improving method to improve extracted compound yield, and isolation of targeted compound. This phase is also important to assess whether the presence of harmful compound in a plant.

Literature shows various phytochemical compounds (such as saponins, flavonoids, and alkaloids) were identified from the extraction of different bamboo part. Bamboo leaves, culms, and root considered as favored part of most research contribution compared to bamboo rhizome. The inadequacy of available information regarding bamboo rhizome instead of numerous studies of another bamboo part probably due to easier harvesting procedure, higher biomass per plant, and higher in concentration of targeted functional properties. Tanaka *et al.* [15] reported that the extracts of *Phyllostachys pubescens* rhizome has potential as skin whitening agent, anti-allergy, and potent anti-oxidant activity. On the other hand, rhizome from various rhizomatous plants has been employed as a source for medicinal remedies and being analyzed for their potent functional properties [16-20].

Moreover, the ontogeny age-related and plant development phase were reported as one of determinant factor on phytochemical compound composition and concentration in *Fargesia rufa* and *Fargasia scabrida* bamboo species [21],and in other plant species also [22-26].Several studies on natural stand such as *Anemopsis californica* [27],*F. rufa* and *F. scabrida* [21], *Phyllanthus amarus* [28],and *Quillaja saponaria* [29] species found that different environmental condition also greatly affect the variation of phytochemical composition and concentration per se.

Though, this study was hypothesized a great variation of phytochemical compound and concentration may present in *G. scortechinii* rhizome regarding different study sites and rhizome ages. This study attempts to assess beneficial phytochemical compounds of *G. scortechinii* rhizome and provide preliminary information contribute to plant-derived biomaterials.

MATERIALS AND METHODS

Sampling

Samples of *G. scortechinii* rhizome were collected at three different locations in Peninsular Malaysia: (a) 5°37’12.46” N, 101°38’51.09” E, Amanjaya Forest Reserve (FR), Perak, elevation ~700 m above sea level, average annual precipitation 2000 mm; (b) 3°10’50.93” N, 101°58’37.60” E, Compartment 189, Kenaboi FR, Negeri Sembilan, elevation 320 m above sea level, average annual precipitation 2100 mm; and (c) 3°0’16.17” N, 101°38’36.08” E, Compartment 15, Ayer Hitam FR, Selangor, elevation 300 m above sea level, average annual precipitation 2100 mm. All study sites experienced different disturbance activity, e.g. active forest production and wildlife (elephant) habitat at Amanjaya FR, extreme recreation activity (four-wheel drive vehicles) at Kenaboi FR, and education and research activities at Ayer Hitam FR. The soil series were classified as Bukit Temiang, Beserah, and Serdang series respectively.

Samples were collected using selective random sampling method from healthy clumps under natural stand conditions at Amanjaya FR and Kenaboi FR, and planted bamboo stands at Ayer Hitam FR. Clumps that are not fertile or congested were excluded. In each sample, three clumps were selected and used as three replicates. Four consecutive rhizomes (Figure 1) from each clump—i.e., new sprouts (estimated age of < one month), young rhizomes (estimated age - one year), pre-mature rhizomes (estimated age - two years), and mature rhizomes (estimated age - three years)—were selected [30]. The estimation of ages is according to characters of culms [31] and rhizome [32].

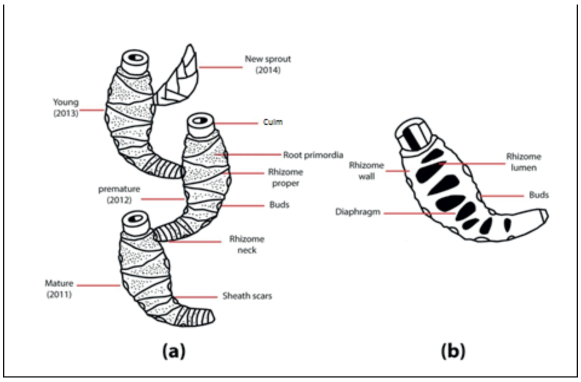


Figure 1. Diagram of (a) four consecutive *Gigantochloa scortechinii* rhizomes, and (b) transverse section of rhizome.

Sample Preparation

Fresh rhizome was peeled, sliced and dried under shade at room temperature until reached a constant weight. Dried sample from each replicate was composited, ground and pulverized to powder with Ring-Knife Flaker, PZ 8, Pallmann Maschinenfabrik. Homogenized sample was collected and passed through a 250 µm sieve using Endecotts Test Sieve Shaker and stored in the sealed plastic bag in an airtight container until further analysis. The air-dried state sample generally does not affect present of inherent phytochemical such as saponin, general glycosides, coumarins, and cyanogenic glycosides [33].

Solvent Extraction

20 g of homogenized sieve dried sample were extracted using 200 ml solvent extraction (70 ethanol: 30 water) with soxhlet apparatus and evaporated using rotary evaporator at 40 °C [34]. Ethanol was selected instead of water, acetone, chloroform, and ether because more efficient in cell wall degradation, preserve several enzymes from degraded and easier to penetrate the cellular membrane. Ethanol also much more suitable instead of methanol due to toxicity level of methanol, and by adding 30 % of water was to increase the solvent’s polarity [35-36].

Phytochemical Compounds Analysis

Ethanolic extracts of *G. scortechinii* was analyzed using Gas Chromatography-Mass Spectrometry, GCMS-QP2010 Ultra, Shimadzu, at Spectroscopy Unit, Institute of Bioscience, Universiti Putra Malaysia. The operating parameters used were: capillary column BPX5 5 % phenyl methyl siloxane (30m x 0.25mm i.d. x 0.25µm film thickness), split injection mode with pressure 37.1kPa. The temperature was set initially at 50°C and raised up to 250°C at 10°C min-1 until completed. The Flavors and Fragrances of Natural and Synthetic Compound (FFNSC) 1.3, National Institute of Standards and Technology (NIST) 11, Pfleger-Maurer-Waber-Drugs-and-Pesticides-Library for Toxicology (PMW_tox2), and Wiley229 spectral library were used to identify the detected compounds.

Statistical Analysis

The effect of different study sites and rhizome ages on phytochemical concentration was analyzed using either one-way analysis of variance or one-sample t-test. Two-way analysis of variance was not used in the analysis due to no replication of the sample (only one homogenized sample) and most of the identified phytochemical composition did not meet the assumption requirement regarding statistical procedure. The statistical analysis was conducted using IBM SPSS statistics version 21.0.

RESULTS AND DISCUSSION

GCMS chromatogram (Figure 2, 3, and 4) indicate 38, 27, 29, 30, 14, 30, 30, 29, 30, 29, 26, and 30 phytochemical compounds respectively detected in the ethanolic extracts of *G. scortechinii* rhizome at different study sites and rhizome ages. A total of 56 compounds were identified and the detailed of compounds number, name of the compound, molecular formula, and molecular weight of identified compounds are shown in Table 1. The composition and concentration of identified compounds at each study site and age are shown in Table 2, 3, and 4.

Variation among Study Sites

The composition of the phytochemical compound at different study sites were found differ from each other (Table 5). Regardless rhizome age, highest number of identified compounds were found at Amanjaya FR (41 compounds) followed by Kenaboi FR (33 compounds) and Ayer Hitam FR (30 compounds). Some compounds found at either only one or two study sites. For examples, Compound no. 5, 17, 35, 45, 48, 50, 51, 52, 53, 54, and 55 were found only at Amanjaya FR, while Compound no. 9, 16, 19, 27, 41, and 46 were found only at Kenaboi FR. Furthermore, six compounds were found only at Amanjaya FR and Kenaboi FR such as Compound no. 1, 23, 36, 40, 49, and 56. Three compounds were only found at both Kenaboi FR and Ayer Hitam FR such as Compound no. 4, 20, and 37. Three compounds were only found at Amanjaya FR and Ayer Hitam FR such as Compound no. 8, 34, and 44.

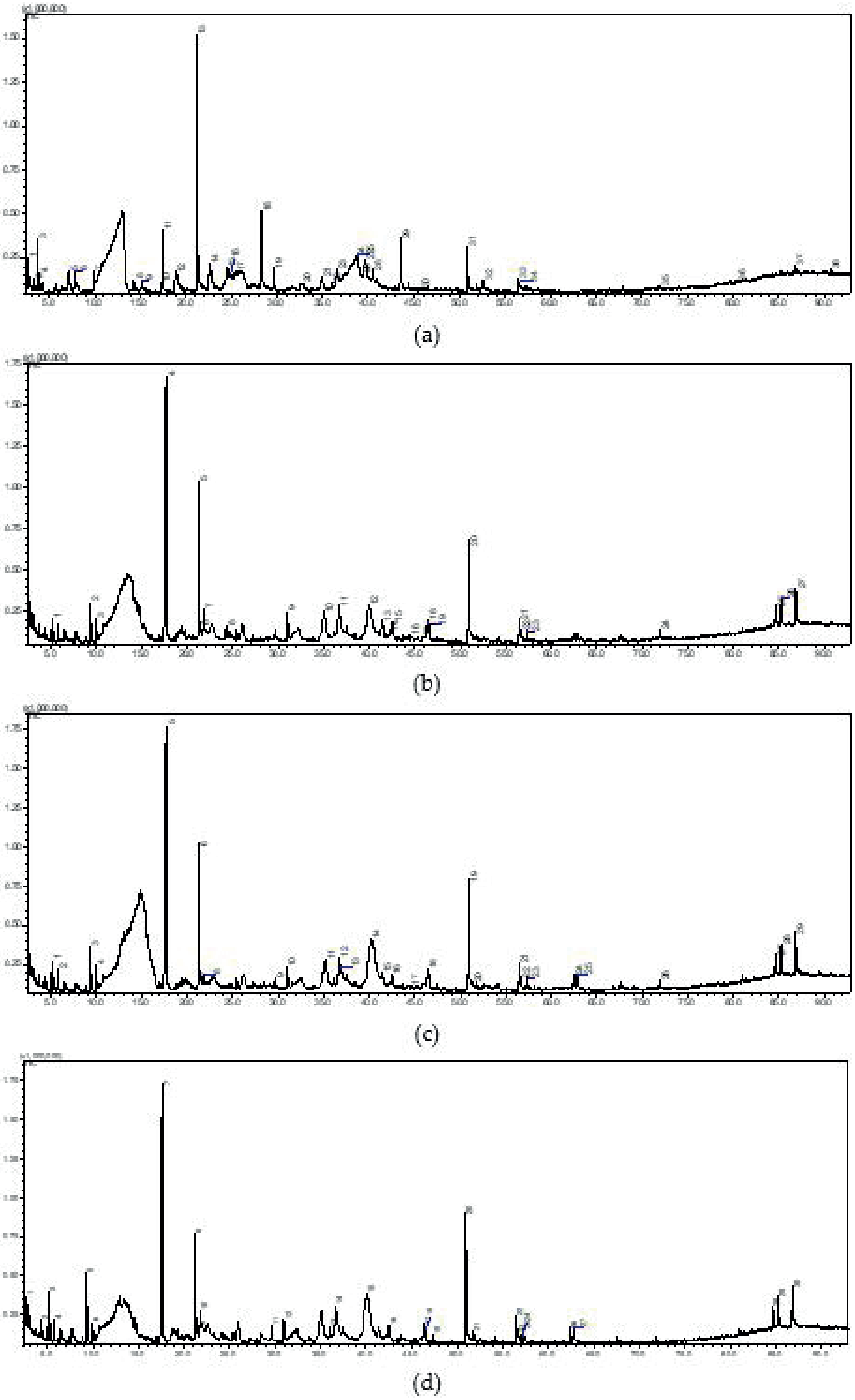


Figure 2. GC-MS chromatogram of ethanolic extract of *Gigantochloa scortechinii* rhizome at Amanjaya Forest Reserve showed peaks of the test compound *versus* retention time in minutes; (a) new sprout, (b) young, (c) pre-mature, and (d)

mature rhizome.

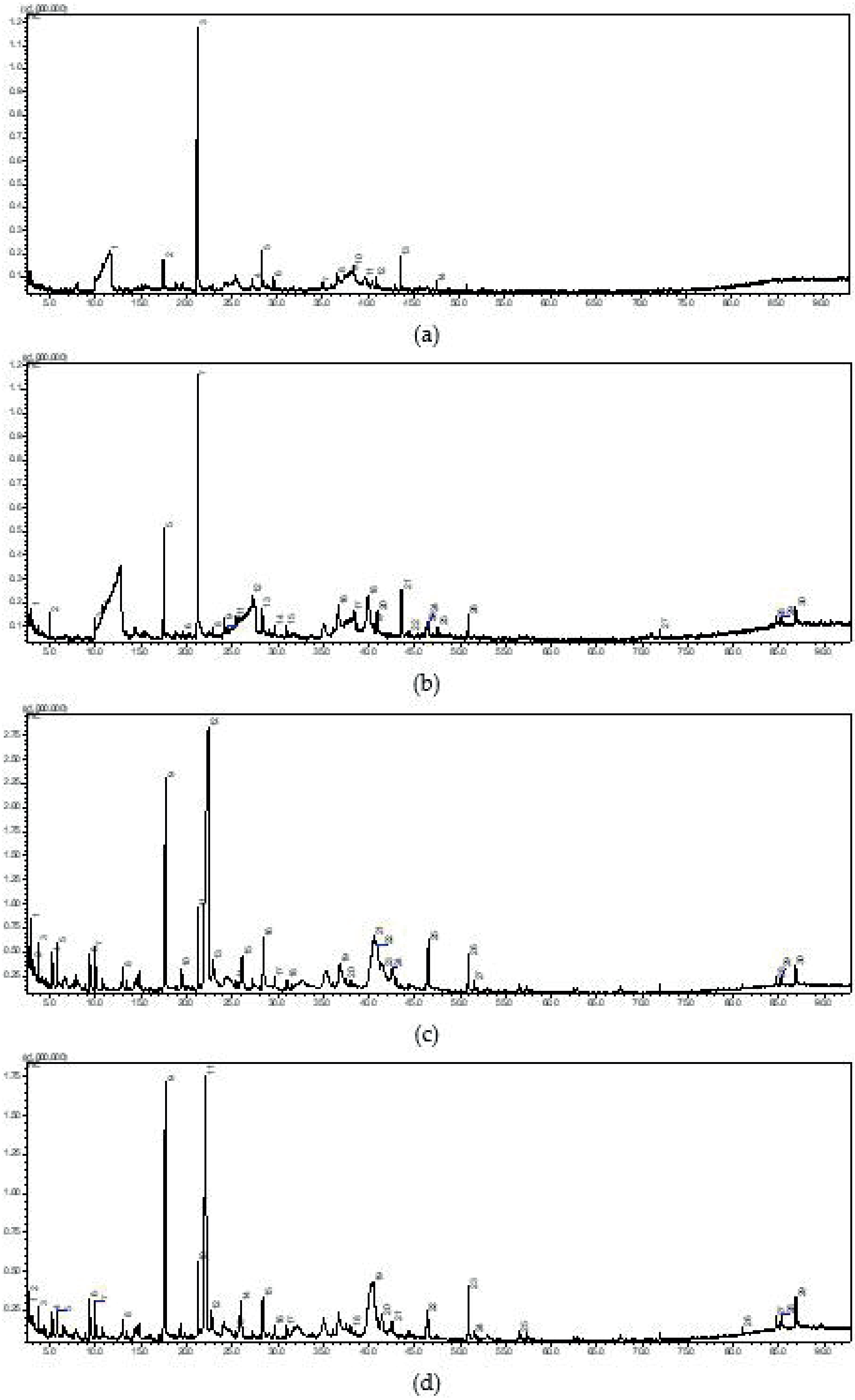
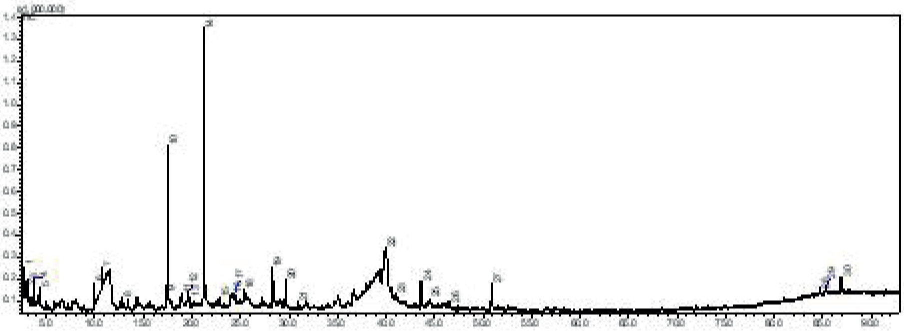
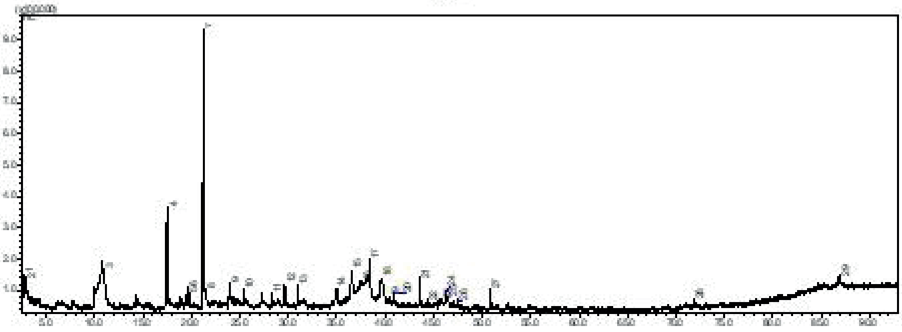


Figure 3. GC-MS chromatogram of ethanolic extract of *Gigantochloa scortechinii* rhizome at Kenaboi Forest Reserve showed peaks of the test compound *versus* retention time in minutes; (a) new sprout, (b) young, (c) pre-mature, and (d) mature rhizome.



(a)



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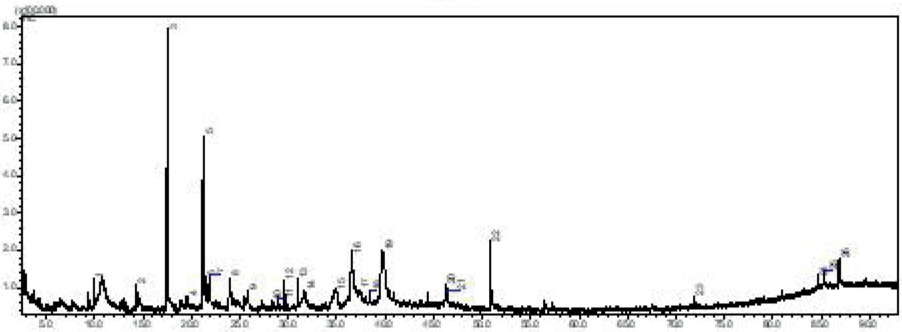

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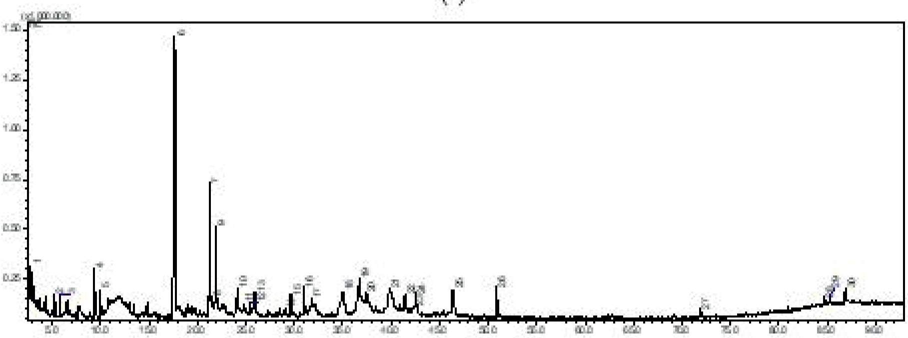

(d)

Figure 4. GC-MS chromatogram of ethanolic extract of *Gigantochloa scortechinii* rhizome at Ayer Hitam Forest Reserve showed peaks of the test compound *versus* retention time in minutes; (a) new sprout, (b) young, (c) pre-mature, and (d) mature rhizome.

Table 1. Identification Details of Phytochemical Compounds

Compound No.	Compound	Molecular formula	Molecular weight
1	Butanal, 2-Methyl-	$C_5H_{10}O$	86.132
2	2-Propanone, 1-Hydroxy-	$C_3H_6O_2$	74.079
3	2-Propenoic acid (CAS) Acrylic acid	$C_3H_4O_2$	72.063
4	Ethylene Glycol P5	$C_2H_6O_2$	62.068
5	Glycerin	$C_3H_8O_3$	92.094
6	2-Propyn-1-Ol (CAS) Propargyl Alcohol	C_3H_4O	56.063
7	Acetol	$C_3H_6O_2$	74.079
8	2,3-Butanediol, [R-(R*,R*)]-	$C_4H_{10}O_2$	90.121
9	Urethane	$C_3H_7NO_2$	89.093
10	Furfural	$C_5H_4O_2$	96.084
11	2-Furanmethanol (CAS) Furfuryl Alcohol	$C_5H_6O_2$	98.100
12	Isoamylbutyrate	$C_9H_{18}O_2$	158.241
13	2-Furancarboxaldehyde, 5-Methyl- (CAS) Furfural <5-Methyl->	$C_6H_6O_2$	110.111
14	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	$C_6H_8O_4$	144.125
15	N,N'-Dimethylpiperazine	$C_6H_{14}N_2$	114.189
16	Pentanoic Acid, 4-Oxo-	$C_5H_8O_3$	116.116
17	1,3,5-Triazine-2,4,6-Triamine	$C_3H_6N_6$	126.120
18	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	$C_6H_8O_4$	144.125
19	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	$C_6H_6O_4$	142.109
20	5-Methoxypyrrolidin-2-One	$C_5H_9NO_2$	115.132
21	Benzofuran, 2,3-Dihydro-	C_8H_8O	120.149
22	Hydroxy Methyl Furfural	$C_6H_6O_3$	126.110
23	1,2,3-Propanetriol, 1-Acetate	$C_5H_{10}O_4$	134.131
24	Malic Acid	$C_4H_6O_5$	134.087
25	Butanedioic Acid, Hydroxy-, Diethyl Ester, (.+/-.)-	$C_8H_{14}O_5$	190.194
26	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	150.175
27	Phenol, 2,6-Dimethoxy- (CAS) 2,6-Dimethoxyphenol	$C_8H_{10}O_3$	154.163
28	Benzaldehyde, 4-Hydroxy- (CAS) P-Hydroxybenzaldehyde	$C_7H_6O_2$	122.121
29	Formyl Glutamine	$C_9H_{14}N_2O_5$	230.218
30	L-Proline, 5-Oxo-, Methyl Ester	$C_6H_9NO_3$	143.141
31	Benzaldehyde, 4-Hydroxy-3-Methoxy- (CAS) Vanillin	$C_8H_8O_3$	152.147
32	Phenol, 3,4-Dimethoxy-	$C_8H_{10}O_3$	154.163
33	Benzoic acid, 4-Hydroxy-, Propyl Ester (CAS) N-Propyl P-Hydroxybenzoate	$C_{10}H_{12}O_3$	180.203
34	Salicylic Acid <Para->	$C_7H_6O_3$	138.122
35	Dodecanoic Acid (CAS) Lauric Acid	$C1_2H_{24}O_2$	200.318
36	Benzoic Acid, 4-Hydroxy-3-Methoxy-	$C_8H_8O_4$	168.147
37	Phenol, 3,4,5-Trimethoxy-	$C_9H_{12}O_4$	184.189
38	L-Arabinitol	$C_5H_{12}O_5$	152.146
39	Quinic Acid	$C_7H_{12}O_6$	192.167
40	Benzaldehyde, 4-Hydroxy-3,5-Dimethoxy- (CAS) Syringaldehyde	$C_9H_{10}O_4$	182.173
41	Ethyl 4-Hydroxy-Dl-Mandelate	$C_{10}H_{12}O_4$	196.202
42	Coniferyl Alcohol	$C_{10}H_{12}O_3$	180.203
43	Benzoic Acid, 4-Hydroxy-3,5-Dimethoxy-	$C_9H_{10}O_5$	198.173
44	Ethanone, 1-(4-Hydroxy-3,5-Dimethoxyphenyl)-	$C_{10}H_{12}O_4$	196.200
45	Pentadecanoic Acid	$C_{15}H_{30}O_2$	242.398

46	Ethyl Ester Vanillylmandelic Acid .BETA.-O-Ethyl Ether	$C_{13}H_{18}O_5$	254.000
47	N-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256.424
48	Hexadecanoic Acid, Ethyl Ester (CAS) Ethyl Palmitate	$C_{18}H_{36}O_2$	284.477
49	9,12-Octadecadienoic Acid (Z,Z)-	$C_{18}H_{32}O_2$	280.446
50	Hexadec-(9Z)-Enal	$C_{16}H_{30}O$	238.415
51	Cis-Vaccenic Acid	$C_{18}H_{34}O_2$	282.468
52	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$	210.356
53	Linoleic Acid Ethyl Ester	$C_{20}H_{36}O_2$	308.499
54	Octadecanoic Acid	$C_{18}H_{36}O_2$	284.477
55	Stigmasterol	$C_{29}H_{48}O$	412.691
56	Stigmast-5-En-3-Ol, (3.Beta.)- (CAS) 24.Beta.-Ethyl-5.Delta.-Cholesten-3.Beta.-Ol	$C_{29}H_{50}O$	414.707

Table 2. Concentration of Phytochemical Compounds of Four Rhizome Ages at Amanjaya Forest Reserve

Compound No.	Retention time (min)	Concentration (%)			
		New Sprout	Young rhizome	Pre-Mature Rhizome	Mature Rhizome
1	2.59±0.00	nd	nd	nd	0.56
2	2.71±0.00	0.57	nd	nd	nd
5	3.29±0.00	0.48	nd	nd	nd
6	3.68±0.00	2.19	nd	nd	nd
7	3.96±0.00	1.35	nd	nd	nd
8	4.36±0.00	nd	nd	nd	0.49
10	5.22±0.01	nd	1.01	1.37	1.91
11	5.71±0.01	nd	nd	0.84	1.08
12	7.76±0.00	1.26	nd	nd	nd
13	9.34±0.01	nd	2.98	2.94	4.95
14	9.95±0.02	1.09	1.30	1.33	0.96
17	14.29±0.00	0.95	nd	nd	nd
18	17.59±0.05	5.10	26.20	24.09	27.22
21	21.22±0.01	23.09	10.31	9.46	7.64
22	21.84±0.01	nd	1.97	0.71	2.45
23	22.63±0.00	6.82	nd	nd	nd
24	24.29±0.00	nd	1.79	nd	nd
26	25.42±0.00	0.59	nd	nd	nd
28	28.29±0.00	9.04	nd	nd	nd
31	29.61±0.00	1.85	nd	0.69	1.17
32	30.98±0.01	nd	2.54	1.67	1.97
34	35.02±0.04	2.88	6.56	nd	nd
35	36.05±0.02	0.56	nd	nd	0.56
36	36.73±0.01	nd	nd	3.24	6.76
38	38.84±0.00	2.61	nd	nd	nd
39	40.03±0.33	4.23	8.95	17.07	7.44
40	40.50±0.00	0.80	nd	nd	nd
42	43.59±0.00	5.55	nd	nd	nd
43	46.34±0.02	nd	1.67	1.65	1.25
44	46.49±0.01	nd	1.02	nd	0.83
45	47.38±0.00	nd	nd	nd	0.33
47	50.90±0.04	4.03	8.05	7.81	11.52

48	51.74±0.00	nd	nd	nd	0.59
49	56.43±0.01	1.15	1.72	1.57	1.91
50	56.59±0.00	1.23	nd	nd	nd
51	56.58±0.00	nd	nd	1.45	nd
52	56.60±0.00	nd	1.40	nd	nd
53	57.11±0.00	nd	nd	nd	0.36
54	57.30±0.01	nd	0.79	0.92	0.93
55	85.26±0.00	nd	2.52	nd	3.08
56	86.84±0.01	nd	3.80	3.90	nd

Note: nd = not detected

Table 3. Concentration of Phytochemical Compounds of Four Rhizome Ages at Kenaboi Forest Reserve

Compound No.	Retention time (min)	Concentration (%)			
		New Sprout	Young rhizome	Pre-Mature Rhizome	Mature Rhizome
1	2.59±0.00	nd	nd	nd	0.13
2	2.79±0.04	nd	nd	0.50	0.33
4	3.01±0.04	nd	0.72	0.19	nd
6	3.75±0.03	nd	nd	0.80	0.44
9	4.98±0.00	nd	1.04	nd	nd
10	5.22±0.00	nd	nd	2.11	1.25
11	5.73±0.02	nd	nd	1.51	0.92
13	9.35±0.00	nd	nd	2.52	2.34
14	9.96±0.01	nd	1.46	1.45	1.66
16	12.95±0.00	nd	nd	nd	1.18
18	17.59±0.13	3.34	9.70	20.64	19.81
19	19.45±0.00	nd	nd	1.69	nd
20	19.59±0.00	nd	0.75	nd	nd
21	21.22±0.01	32.04	24.67	3.59	3.53
22	22.25±0.11	nd	nd	32.63	27.05
23	22.82±0.09	nd	nd	2.23	1.91
24	24.12±0.00	nd	2.27	nd	nd
26	25.43±0.00	nd	0.96	0.25	0.26
27	27.23±0.01	1.47	9.92	nd	nd
28	28.34±0.05	7.27	3.24	3.91	2.78
31	29.64±0.01	1.64	1.51	0.60	0.61
32	31.00±0.03	nd	1.91	0.55	0.69
36	36.74±0.09	nd	5.22	2.22	nd
37	38.42±0.00	nd	1.01	nd	nd
39	40.42±0.49	nd	12.07	2.06	18.41
40	40.54±0.00	nd	nd	3.95	nd
41	40.92±0.00	1.79	2.56	nd	nd
42	43.60±0.00	4.83	4.85	nd	nd
43	46.41±0.10	nd	2.01	3.64	2.01
46	47.49±0.01	1.38	1.28	nd	nd
47	50.88±0.02	nd	2.32	1.90	2.70
49	56.43±0.00	nd	nd	nd	0.45
56	86.86±0.01	nd	nd	1.26	2.00

Note: nd = not detected

Table 4. Concentration of Phytochemical Compounds of Four Rhizome Ages at Ayer Hitam Forest Reserve

Compound No.	Retention time (min)	Concentration (%)			
		New Sprout	Young rhizome	Pre-Mature Rhizome	Mature Rhizome
2	2.77±0.03	0.70	0.72	nd	0.60
3	2.89±0.01	0.30	1.07	nd	nd
4	3.00±0.00	0.68	nd	nd	nd
6	3.72±0.00	0.82	nd	nd	nd
8	4.34±0.00	0.70	nd	nd	nd
10	5.24±0.00	nd	nd	nd	1.20
11	5.75±0.00	nd	nd	nd	0.92
13	9.37±0.00	nd	nd	nd	3.90
14	9.97±0.02	1.42	nd	1.88	1.74
15	12.72±0.00	0.82	nd	nd	nd
18	17.54±0.07	15.89	10.59	23.77	29.30
20	19.57±0.06	2.79	nd	1.27	nd
21	21.22±0.02	22.39	28.54	13.02	9.28
22	21.87±0.04	nd	nd	2.56	7.21
24	24.06±0.13	2.14	4.06	4.69	4.87
25	24.82±0.00	nd	nd	nd	1.36
26	25.44±0.01	1.04	1.60	nd	0.79
28	28.31±0.02	4.13	1.72	0.23	nd
29	28.94±0.00	nd	nd	1.29	nd
30	29.07±0.00	nd	nd	nd	0.66
31	29.63±0.02	2.26	2.13	2.13	1.71
32	30.98±0.03	nd	2.51	2.17	2.56
33	34.98±0.00	nd	nd	0.97	nd
34	34.99±0.00	nd	3.39	nd	nd
37	38.42±0.00	nd	nd	1.02	nd
39	39.83±0.16	14.15	8.48	8.86	5.29
42	43.61±0.00	2.66	3.58	nd	nd
43	46.29±0.04	nd	1.58	1.76	1.53
44	46.48±0.00	nd	nd	1.54	nd
47	50.86±0.01	2.68	2.28	5.26	2.29

Note: nd = not detected

Table 5. Statistical Significances of the Responses of Phytochemical Compounds with Study Site

Compound No.	Concentration (%)			P-value and statistical significance
	Amanjaya FR	Kenaboi FR	Ayer Hitam FR	
1	0.56±nc	0.13±nc	nd	0.355nsb
2	0.57±nc	0.42±0.09	0.67±0.04	0.104ns a
3	nd	nd	0.69±0.39	nc
4	nd	0.46±0.27	0.68±nc	0.710ns a
5	0.48±nc	nd	nd	nc
6	2.19±nc	0.62±0.18	0.82±nc	0.190ns a
7	1.35±nc	nd	nd	nc

8	0.49±nc	nd	0.70±nc	0.111ns b
9	nd	1.04±nc	nd	nc
10	1.43±0.26	1.68±0.43	1.20±nc	0.749ns a
11	0.96±0.12	1.22±0.30	0.92±nc	0.699ns a
12	1.26±nc	nd	nd	nc
13	3.62±0.66	2.43±0.09	3.90±nc	0.407ns a
14	1.17±0.09	1.52±0.07	1.68±0.14	0.019*a
15	nd	nd	0.82±nc	nc
16	nd	1.18±nc	nd	nc
17	0.95±nc	nd	nd	nc
18	20.65±5.22	13.37±4.17	19.89±4.14	0.489ns a
19	nd	1.69±nc	nd	nc
20	nd	0.75±nc	2.03±0.76	0.509ns a
21	12.63±3.53	15.96±7.31	18.31±4.39	0.757ns a
22	1.71±0.52	29.84±2.79	4.89±2.33	0.001**a
23	6.82±nc	2.07±0.16	nd	0.037*a
24	1.79±nc	2.27±nc	3.94±0.62	0.337ns a
25	nd	nd	1.36±nc	nc
26	0.59±nc	0.49±0.24	1.14±0.24	0.247ns a
27	nd	5.70±4.23	nd	nc
28	9.04±nc	4.30±1.02	2.03±1.14	0.072ns a
29	nd	nd	1.29±nc	nc
30	nd	nd	0.66±nc	nc
31	1.24±0.34	1.09±0.28	2.06±0.12	0.045*a
32	2.06±0.26	1.05±0.43	2.41±0.12	0.042*a
33	nd	nd	0.97±nc	nc
34	4.72±1.84	nd	3.39±nc	0.748ns a
35	0.56±0.00	nd	nd	nc
36	5.00±1.76	3.72±1.50	nd	0.636ns a
37	nd	1.01±nc	1.02±nc	0.003**b
38	2.61±nc	nd	nd	nc
39	9.42±2.73	10.85±4.76	9.20±1.84	0.925ns a
40	0.80±nc	3.95±nc	nd	0.373ns b
41	nd	2.18±0.39	nd	nc
42	5.55±nc	4.84±0.01	3.12±0.46	0.079ns a
43	1.52±0.14	2.55±0.54	1.62±0.07	0.123ns a
44	0.93±0.10	nd	1.54±nc	0.166ns a
45	0.33±nc	nd	nd	nc
46	nd	1.33±0.05	nd	nc
47	7.85±1.53	2.32±0.23	3.13±0.72	0.014*a
48	0.59±nc	nd	nd	nc
49	1.59±0.16	0.45±nc	nd	0.051ns a
50	1.23±nc	nd	nd	nc
51	1.45±nc	nd	nd	nc
52	1.40±nc	nd	nd	nc
53	0.36±nc	nd	nd	nc
54	0.88±0.05	nd	nd	nc
55	2.80±0.28	nd	nd	nc

56	3.85±0.05	1.63±0.37	nd	0.027*a

Note: ± = standard error, * = significant at p<0.05, ** = significant at p<0.01, ns = not significant different, nd = not detected, nc = not computed, a = statistical using One-Way ANOVA, b = statistical using One-Sample T Test

Table 6. Statistical Significances of the Responses of Phytochemical Compounds with Rhizome Age

Compound No.	Concentration (%)				P-value and statistical significance
	New Sprout	Young rhizome	Pre-Mature Rhizome	Mature Rhizome	
1	nd	nd	nd	0.35±0.22	nc
2	0.64±0.07	0.72±nc	0.50±nc	0.47±0.14	0.577ns a
3	0.30±nc	1.07±nc	nd	nd	0.326ns b
4	0.68±nc	0.72±nc	0.19±nc	nd	0.09ns b
5	0.48±nc	nd	nd	nd	nc
6	1.51±0.69	nd	0.80±nc	0.44±nc	0.725ns a
7	1.35±nc	nd	nd	nd	nc
8	0.70±nc	nd	nd	0.49±nc	0.111ns b
9	nd	1.04±nc	nd	nd	nc
10	nd	1.01±nc	1.74±0.37	1.45±0.23	0.490ns a
11	nd	nd	1.18±0.34	0.97±0.05	0.493ns a
12	1.26±nc	nd	nd	nd	nc
13	nd	2.98±nc	2.73±0.21	3.73±0.76	0.625ns a
14	1.26±0.17	1.38±0.08	1.55±0.17	1.45±0.25	0.774ns a
15	0.82±nc	nd	nd	nd	nc
16	nd	nd	nd	1.18±nc	nc
17	0.95±nc	nd	nd	nd	nc
18	8.11±3.92	15.50±5.36	22.83±1.10	25.44±2.88	0.039*a
19	nd	nd	1.69±nc	nd	nc
20	2.79±nc	0.75±nc	1.27±nc	nd	0.120ns b
21	25.84±3.11	21.17±5.55	8.69±2.75	6.82±1.71	0.013*a
22	nd	1.97±nc	11.97±10.35	12.24±7.53	0.842ns a
23	6.82±nc	nd	2.23±nc	1.91±nc	0.148ns b
24	2.14±nc	2.71±0.69	4.69±nc	4.87±nc	0.410ns a
25	nd	nd	nd	1.36±nc	nc
26	0.82±0.23	1.28±0.32	0.25±nc	0.53±0.27	0.286ns a
27	1.47±nc	9.92±nc	nd	nd	0.406ns b
28	6.81±1.44	2.48±0.76	2.07±1.84	2.78±nc	0.203ns a
29	nd	nd	1.29±nc	nd	nc
30	nd	nd	nd	0.66±nc	nc
31	1.92±0.18	1.82±0.31	1.14±0.50	1.16±0.32	0.328ns a
32	nd	2.32±0.21	1.46±0.48	1.74±0.55	0.423ns a
33	nd	nd	0.97±nc	nd	nc
34	2.88±nc	4.98±1.59	nd	nd	0.585ns a
35	0.56±nc	nd	nd	0.56±nc	nc
36	nd	5.22±nc	2.73±0.51	6.76±nc	0.205ns a
37	nd	1.01±nc	1.02±nc	nd	0.003**b
38	2.61±nc	nd	nd	nd	nc
39	9.19±4.96	9.83±1.13	9.33±4.34	10.38±4.06	0.996ns a
40	0.80±nc	nd	3.95±nc	nd	0.373ns b

41	1.79±nc	2.56±nc	nd	nd	0.112ns b
42	4.35±0.87	4.22±0.64	nd	nd	0.921ns a
43	nd	1.75±0.13	2.35±0.65	1.60±0.22	0.428ns a
44	nd	1.02±nc	1.54±nc	0.83±nc	0.034*b
45	nd	nd	nd	0.33±nc	nc
46	1.38±nc	1.28±nc	nd	nd	0.024*b
47	3.36±0.68	4.22±1.92	4.99±1.71	5.50±3.01	0.921ns a
48	nd	nd	nd	0.59±nc	nc
49	1.15±nc	1.72±nc	1.57±nc	1.18±0.73	0.956ns a
50	1.23±nc	nd	nd	nd	nc
51	nd	nd	1.45±nc	nd	nc
52	nd	1.40±nc	nd	nd	nc
53	nd	nd	nd	0.36±nc	nc
54	nd	0.79±nc	0.92±nc	0.93±nc	0.003**b
55	nd	2.52±nc	nd	3.08±nc	0.063ns b
56	nd	3.80±nc	2.58±1.32	2.00±nc	0.818ns a

Note: ± = standard error, * = significant at $p<0.05$, ** = significant at $p<0.01$, ns = not significant different, nd = not detected, nc = not computed, a = statistical using One-Way ANOVA, b = statistical using One-Sample T Test

Results in Table 5 indicate that 18 identified compounds were found at all three study sites. However, the concentration of Compound 14, 31, 32, and 47 were found significant different ($p<0.05$) with study site. The concentration of Compound 21 was found highly significant different ($p<0.01$) with study site. The concentration of the rest 13 compounds (Compound 2, 6, 11, 13, 18, 21, 24, 26, 28, 39, 42, and 43), however, found not significant different ($p<0.05$) with study site. The variation of compound's composition and concentration are believed to be related with the peculiar site condition such as precipitation, soil fertility, and disturbance type. Highest number of compound in Amanjaya FR could also relate with the natural stand condition as elephant's habitat, and resulted to a great plant-animal interaction.

Variation among Rhizome Ages

Different rhizome ages also gave a great variation on phytochemical composition and concentration (Table 6). From a total of 56 compounds, number of compounds found at all four rhizome ages was 31, 32, 31, and 32 compounds in ascending order. Three compounds such as Compound no. 12, 38, and 50 were only found in new sprout, otherwise, seven compounds such as Compound no. 1, 16, 25, 30, 45, 48, and 53 were only found in mature rhizome.

Result (Table 6) also indicates pattern of the compound composition with the changes of rhizome age. Some of the compounds (Compound no. 3, 27, and 34) were found in new sprout and young rhizome but not found after. Some compounds (Compound no. 10, 13, and 32), however, were found from young to mature rhizome but not found before. The irregular pattern of compounds composition were also observed such as for Compound no. 6, 8, 23, and 35 which found in younger and older-age rhizome but not identified in middle-age rhizome.

From 56 identified compounds, results in Table 6 indicate that only 11 compounds were identified at all four rhizome ages. The concentration of both Compound 18 and 21 were found significant different ($p<0.05$) with rhizome age. Compound 18 was found increased with increasing rhizome age (8.11±3.92, 15.50±5.36, 22.83±1.10, and 25.44±2.88 % respectively) while compound 21 was found inversely (25.84±3.11, 21.17±5.55, 8.69±2.75, and 6.82±1.71 % respectively). The concentration of another nine compounds (Compound 2, 14, 24, 26, 28, 31, 39, 47, and 49) were found not significant different with rhizome age.

Variation among Study Sites and Rhizome Ages

Regarding study site and rhizome age, although a great variation of compound composition and concentration has been observed, Compound no. 18 and 21 were found at all three study sites and four rhizome ages (Table 2, 3, and 4).

Compound no. 18 (4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-) (DDMP) was found higher at Amanjaya FR (20.65±5.22 %) followed by Ayer Hitam FR (19.89±4.14 %) and Kenaboi FR (13.37±4.17 %)(Table 5). DDMP was also found higher in mature rhizome (25.44±2.88 %) followed by premature (22.83±1.10 %), young (15.50±5.36 %) and new sprout (8.11±3.92 %)(Table 6). DDMP or pyranone is a flavonoid fraction which important potent exhibited fungal activity, strong antioxidant, and radical scavenging activity [37]. DDMP in the ethanolic extract of mature *G. scortechinii* rhizome (19.81 – 29.30 %) in present study is higher in concentration compared in methanolic extract of medicinal herb (*Clerodendrum viscosum*) leaves (4.19 %) [38], and *Nerium oleander* stems (4.0 %) [39] in previous studies.

Compound no. 21 (Benzofuran, 2,3-dihydro-) also known as coumaran was found higher at Ayer Hitam FR (18.31±4.39 %) follower by Kenaboi FR (15.96±7.31 %) and Amanjaya FR (12.63±3.53 %)(Table 6). Its concentration was also found higher in new sprout (25.84±3.11 %) followed by young (21.17±5.55 %), pre-mature (8.69±2.75 %), and mature rhizome (6.82±1.71 %)(Table 5). This compound found potent pharmaceutical activities, such as improving cardiovascular system and anti-allergic activities. Derivatives of coumaran found potent antibacterial, antifungal, and potent exhibit *Staphylococcus aureus* and *Escherichia coli activities* [40]. The concentration of coumaran present in new sprout of *G. scortechinii* rhizome (22.39 – 32.04 %) in present study is greatly higher than that found in the methanol extract of *N. orleander* (traditionally used for various illness in China and India) leaves (13.73 %) and stems (4.0 %) [39] in previous study.

Compound no. 32 and 43 were found in all three older-ages rhizomes (from young to mature rhizome) but not identify in new sprout at all three study sites (Table 2, 3, and 4). The concentration of Compound no. 32 was found significant different ($p<0.05$) while Compound no. 43 was found not significant different ($p<0.05$) with study site (Table 5). Both compounds were found not significant different in concentration with rhizome ages (from young to mature rhizome) (Table 6).

Compound no. 32 (Phenol,3,4-dimethoxy-) was found as minor compound (0.55 – 2.56 %) in the ethanolic extract at all study sites. This compound is a veratric acid [41]; a phenolic compound with yellow crystalline needle structure; which found most potent antioxidant among phenolic compounds [42], and potent adult beetles (*Acalymma vitatum*) antifeedant with up to 100 % feeding inhibition [43]. Phenolic compounds are important to produce synthetic fibers, fuels, and pesticides, as by-products. This compound was reported found in the extracts of *Anguilla japonica*, *Sphyraena japonica*, and *Engraulis japonicus*'s bacterial fermentation broth [42]. In present study, phenol, 3,4-dimethoxy- concentration was found higher in the ethanolic extract of young *G. scortechinii*'s rhizome (1.91 – 2.54 %) compared to concentration in pyrolysis bio-oil of Straw (0.46 %) and Bagasse (1.44 %) lignin in previous study [44].

Compound no. 43 (Benzoic acid, 4-hydroxy-3,5-dimethoxy-) (HDMBA) was found as minor compound at all three study sites (1.25 – 3.64 %). HDMBA or syringic acid is one of the hydroxyl benzoic acid derivatives which is a phenolic compound with colorless crystallized needle structure [45]. It found potent antioxidant [46], strong anti-coagulation and platelet aggregation inhibitory activities [47], potent anti-inflammation, anti-aging, anti-cancer and anti-heart disease [48].

Furthermore, Compound no. 39 (Quinic acid) (QA) and Compound no. 47 (n-hexadecanoic acid) were found at all three study sites in all four rhizome ages except in new sprout at Kenaboi FR (Table 2, 3 and 4). QA was found not significant different while n-hexadecanoic acid was found significant different ($p<0.05$) with study site (Table 5). N-hexadecanoic acid was found higher at Amanjaya FR (7.85±1.53 %) followed by Ayer Hitam FR (3.13±0.72 %) and Kenaboi FR (2.32±0.23 %). Both QA and n-hexadecanoic acid were found not significant different with rhizome age (Table 6).

The concentration of QA was found in range from 2.06 to 18.41 % in the ethanolic extract of rhizome. QA is a flavonoid compound which also known as cyclohexane carboxylic acid; commonly obtained from Cinchona bark and Coffee beans. Application of QA showed treated groups down-regulated hyperglycemia and oxidative stress by up-regulating insulin and C-peptide levels. Application of bi-flavonoids (quercetin and QA) 50mg/kg exhibited maximum inhibition of pro-apoptotic protein Bax expression and therefore demonstrate in ameliorating hyperglycemia, hyperlipidemia, and insulin resistance in diabetic [49].

The n-hexadecanoic acid or so-called palmitic acid (PA) is a fatty acid (FA). It was found in range from 1.90 to 11.52 % in the ethanolic extract of *G. scortechinii* rhizome. PA is a potent inhibitor of phospholipase A2 and anti-inflammatory [50]. Basically, FA is considered as indispensable compounds for the efficiency of medicinal plant, and hence as constitute essential structural elements of biological membranes in all organism [51].

CONCLUSIONS

Both study site and age-related showed a great variation of phytochemical composition and concentration. 18 compounds were found at all three study sites, however, the composition and concentration can be considered as age-dependent. More or less, the phytochemical composition and concentration are suggested to be more affected by age-related effect compared to different study site with regard to variation of compound composition and concentration. The GC-MS analysis of ethanolic extract of *G. scortechinii* rhizome also exposes a large number (56) of phytochemical compounds identified in *G. scortechinii* rhizome which depicted its potential potent bio-activity. Several beneficial phytochemical compounds such as DDMP, 4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, benzofuran,2,3-dihydro-, phenol,3,4-dimethoxy-, HDMBA, QA, and PA were recommended based on their potent functional properties and their availability (at all study sites and rhizome ages) in the extract. Further study on phytochemical compounds with replication of GC-MS identification, isolation, and investigation on its biological activity could significantly improve the finding on phytochemical compounds in bamboo and hence promote the utilization regarding to plant derive bio-material.

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