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Spatial and Age Related Effects on Phytochemical Compounds of Ethanolic Extract of *Gigantochloa scortechinii* Rhizome

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Abstract: This study was designed to assess variation of possible phytochemical compounds in the ethanolic extracts of *Gigantochloa scortechinii* rhizome. Destructive sampling was done by using selective random sampling method on four consecutive rhizomes from healthy clumps and was conducted at two natural forests and one secondary forest (planted). Homogenized sample were extracted using solvent extraction (70% ethanol) method. Ethanolic extracts of *G. scortechinii* rhizome were qualitatively analyzed using GC/MS GC2010 Plus, Shimadzu to determine the composition of phytochemical compounds and identified using FFNSC 1.3, NIST11, PMW_tox2, and Wiley229 spectral library. A qualitative variation was observed with a total of 56 compounds were identified and differentiated between study site and rhizome age. Results revealed that *G. scortechinii* rhizome contains various phytochemical compounds with potential as a plant of phytopharmaceutical importance. This is the first finding on the spatial and age-related effects of phytochemical compounds in a consecutive rhizome and of *G. scortechinii* rhizome specifically.

Keywords: Spatial; age-related; phytochemical; bamboo; rhizome; GC-MS

1. Introduction

Bamboo has been commercialized in bamboo producer countries especially in term of pharmaceutical, cosmeceutical, nutraceutical, functional food and food additives such as from bamboo leaves, bamboo shaving, bamboo skin, tabasheer, bamboo sap, bamboo shoot, bamboo salt, bamboo charcoal and bamboo vinegar since ancient version and recently supported with scientific evident. Some of those countries and species included China (*Bambusa textilis* McClure, *Bambusa breiflora*, *Phyllostachys nigra* and *Phyllostachys pubescens*) [1,2], India (*Bambusa arundinaceae*) [3], Indonesia (*Bambusa vulgaris*) [4], Japan (*P. pubescens*) [5], and Korea (*P. nigra* and *P. pubescens*) [6-8].

Previous studies revealed various phytochemical compound e.g. saponins, flavonoids, alkaloids, terpenoids, tannins, and phenols; from the extraction of the different bamboo part. However, leaves, culms, and root remained as favored part of most contribution compared to rhizome part. This may due to reason where easier to harvest, higher in biomass per plant, and higher in the concentration of targeted functional properties. Leaves, branches, and outer/skin culms, for example, may contain higher flavonols (phenol compound) because of their biosynthesis process stimulated by sunlight [9]. Guozen bamboo leaf essence is one of the examples of several bamboo leaf product that beneficial to regulate blood fat, purifies the blood, strengthen the heart and neural protection. Solary bamboo capsules for example from bamboo culms produced to stimulate collagen synthesis in bone and connective tissue, and also give re-mineralization effect [10].

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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 such as *Barleria prionitis* [11], *Curculigo orchioides* [12], *Curcuma longa* [13,14], *Drynaria quercifolia* [15], *Ligusticum chuanxiong* [16], *Maranta arundanica* [17], *Nervilia aragoana* [18], *Notopterygium forbesii* [19], *Polygonatum odoratum* [20], *Tupistra chinensis* [21], and *Zingiber officinale* [22-24]. Despite, very lack of information from literature upon bamboo's rhizome used for above purposes compared to another bamboo part.

In 2014, [5] reported that the extracts of the rhizome of *P. pubescens* have potential as skin whitening agent, also as skin tanning agent, hair dyes, and as anti-allergy. They found that the ethanol extracts of its rhizome showed melanin-biosynthesis stimulating activity at 60 μg/mL where cell viability (CV) was 118 % and melanin content (MC) was 144 % with no cytotoxicity effects onto B16 melanoma cell through microculture tetrazolium technique (MTT assay). Ethanol extracts of rhizome also showed antioxidant activity (oxygen radical absorbance capacity, ORAC: 0.71 mgTE/mg, superoxide dismutase, SOD: 0.1 Uμg, and ABTS radical decolorization activity, IC_{50}: 171.5 μg/mL). Despite, hot water extracts of rhizome showed melanin-biosynthesis-inhibitory activity at 120, 60 and 20 μg/mL (CVs were 111 %, 123 % and 115 %, and MCs were 88.9 %, 97.3 % and 93.2 % respectively), and showed anti-allergy activity where significantly ($p<0.05$) inhibited the production of immunoglobulin E (IgE) (37.2 % inhibition rate).

Furthermore, the biosynthesis of phytochemicals in plant can be significantly affected by many intrinsic factors such as plant physiological status and mainly dependent on the ontogeny age-related, and also the external factors as such the biotic and abiotic variation, which resulted to vary in phytochemical composition and concentration per se [25]. The age-related and plant development phase was revealed as determinant factor of phytochemical composition and concentration in *Fargesia rufa*, *F. scabrida* bamboo species [26] and other plant species such *Betula pendula*, *B. platyphylla*, *B. resinifera* [27], *Clinacanthus nutans* [28], *Nerium oleander* [29], *Panax quinquefolium* [30], and *Stevia rebaudiana* [31].

Moreover, the manipulation of *Lycopersicon esculentum* growth and development through the controlled environment to modify the concentration of selected phytochemical compound has been studied by [32] and [33]. [33] for example suggested that the application of a moderate salt stress increased lycopene and other antioxidant concentration of *L. esculentum* fruit, however, the variation of compound concentration varies from 34 to 85 % depicted the different cultivar-specific responses. The variation of the concentration of selected phytochemical compound has also been investigated under different fertilizer regime on *Labisia pumila* [34] and different nutrient concentration applied with different planting media on *Fragaria* x *ananassa* [35].

Several studies on natural stand of plant such as *Anemopsis californica* [36], *F. rufa* and *F. scabrida* [26], *Phyllanthus amarus* [37], and *Quillaja saponaria* [38] at different environmental condition such as altitude, precipitation, temperature and soil fertility found a great variation of phytochemical composition and their concentration, however those not elucidate whether the variation occur reflect the environmental factors or the genotype variation. However, previous studies on *Populus tremuloides* [39], *Secale cereal* [40], *Triticum aestivum* [41,42], and *T. turgidum* [43] revealed that the variation of phytochemical composition is significantly influenced by the environmental factor than the genotype variation.

Though, a great variation of phytochemical composition and concentration is expected present in *G. scortechinii* rhizome regarding different study site and age in the present study. With all the contribution, the present study attempts to determine beneficial phytochemical compounds of the rhizome of *G. scortechinii* as the first effort focus on bamboo's rhizome usage contributed to plant-derived biomaterials in Malaysia.

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2. Results and Discussion

GCMS chromatogram (Figure 1, 2, and 3) indicate of 38, 27, 29, 30, 14, 30, 30, 29, 30, 29, 26, and 30 phytochemical compounds respectively present in the ethanolic extracts of *Gigantochloa scortechinii* rhizome at different study sites and rhizome ages. From all of those compounds present, a total of 56 compounds were identified and the detailed of retention time, the name of the compound, molecular formula, and molecular weight of identified compounds showed in Table 1. The concentration of identified compounds for each study site and age showed in Table 2, 3, and 4.

2.1. Variation between study sites

The composition of the phytochemical compound at different study sites were found differ from each other (Table 5), which some compound only present at either only one or two study sites. For examples, glycerin, 2-propanone,1-hydroxy- (cas) acetol, isoamylbutyrate, 1,3,5-triazine-2,4,6-triamine, dodecanoic acid (cas) lauric acid, pentadecanoic acid, hexadecanoic acid,ethyl ester (cas) ethyl palmitate, hexadec-(9z)-enal, cis-vaccenic acid, 7-tetradecenal,(Z)-, linoleic acid ethyl ester, octadecanoic acid and stigmasterol (Rt: 3.29±0.00, 3.96±0.00, 7.76±0.00, 14.29±0.00, 36.05±0.02, 51.74±0.00, 56.59±0.00, 56.58±0.00, 56.60±0.00, 57.11±0.00, 57.30±0.01, and 85.26±0.00 respectively) were only present at Amanjaya FR, while the urethane, pentanoic acid,4-oxo-, 4h-pyran-4-one,3,5-dihydroxy-2-methyl-, phenol,2,6-dimethoxy- (cas) 2,6-dimethoxyphenol, ethyl 4-hydroxy-dl-mandelate, and ethyl ester vanillylmandelic acid.beta.-o-ethyl ether (Rt: 4.98±0.00, 12.95±0.00, 19.45±0.00, 40.92±0.00, and 47.49±0.01 respectively) were only present at Kenaboi FR.

Furthermore, six compound were present only at Amanjaya FR and Kenaboi FR such as butanal,2-methyl-, 1,2,3-propanetriol,1-acetate, benzoic acid,4-hydroxy-3-methoxy-, benzaldehyde,4-hydroxy-3,5-dimethoxy- (cas) syringaldehyde, 9,12-octadecadienoic acid (z,z)-, stigmast-5-en-3-ol, (3.beta.)- (cas) 24.beta.-ethyl-5.delta.-cholesten-3.beta.-ol (Rt: 2.59±0.00, 22.75±0.03, 36.74±0.09, 40.52±0.02, 56.43±0.00, and 86.85±0.02 respectively), three compound were only present at Kenaboi FR and Ayer Hitam FR such as ethylene glycol p5, 5-methoxypyrrolidin-2-one, and phenol,3,4,5-trimethoxy- (Rt: 3.01±0.01, 19.58±0.07, and 38.42±0.00 respectively), and three compound were only present at Amanjaya FR and Ayer Hitam FR such as 2,3-butanediol,[r-(r*,r*)]-, salicylic acid <para->, and ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)- (Rt: 4.35±0.01, 35.01±0.02, and 46.49±0.01 respectively).

There were also observed similarities in the composition of compound present at all three study sites. Results in Table 5 indicates that 18 identified compounds were found similar at all three study sites. However, the concentration of similar compounds present such as the 2,4-dihydroxy-2,5-dimethyl-3(2h)-furan-3-one (Rt: 9.96±0.03), benzaldehyde,4-hydroxy-3-methoxy- (cas) vanillin (Rt: 29.63±0.02), phenol,3,4-dimethoxy- (Rt: 30.99±0.02), and n-hexadecanoic acid (Rt: 50.88±0.00) were found significantly different ($p<0.05$) with study site. The concentration of hydroxy methyl furfural (Rt: 21.96±0.06) was found highly significantly different ($p<0.01$) with study site. The concentration of the rest 13 similar compounds such as 2-propanone,1-hydroxy-, 2-propyn-1-ol (cas) propargyl alcohol, furfural, 2-furanmethanol (cas) furfuryl alcohol, 2-furancarboxaldehyde,5-methyl- (cas) furfural <5-methyl->, 4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, benzofuran,2,3-dihydro-, malic acid, 2-methoxy-4-vinylphenol, benzaldehyde,4-hydroxy- (cas) p-hydroxybenzaldehyde, quinic acid, coniferyl alcohol, benzoic acid,4-hydroxy-3,5-dimethoxy- (Rt: 2.76±0.03, 3.72±0.00, 5.22±0.02, 5.72±0.02, 9.35±0.02, 17.57±0.08, 21.22±0.02, 24.11±0.08, 25.43±0.01, 28.32±0.01, 40.06±0.15, 43.60±0.01, 46.35±0.01 respectively) were found not significantly different at $p<0.05$.

2.2. Variation between rhizome ages

Different rhizome ages also gave a great variation on phytochemical composition and their concentration (Table 6). For example, three compounds such as isoamylbutyrate (Rt: 1.26±0.00), l-arabinitol (Rt: 38.84±0.00), and hexadec-(9z)-enal (Rt: 56.59±0.00) were present only in new sprout, otherwise, seven compounds such as butanal,2-methyl- (Rt: 2.59±0.00), pentanoic acid,4-oxo- (Rt: 12.95±0.00), butanedioic acid,hydroxy-,diethyl ester, (.+/-.)- (Rt: 24.82±0.00), l-proline,5-oxo methyl

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ester- (Rt: 29.07±0.00), pentadecanoic acid (Rt: 47.38±0.00), hexadecanoic acid ethyl ester (CAS) ethyl palmitate (Rt: 51.74±0.00), and linoleic acid ethyl ester (Rt: 57.11±0.00) were only present in the mature rhizome.

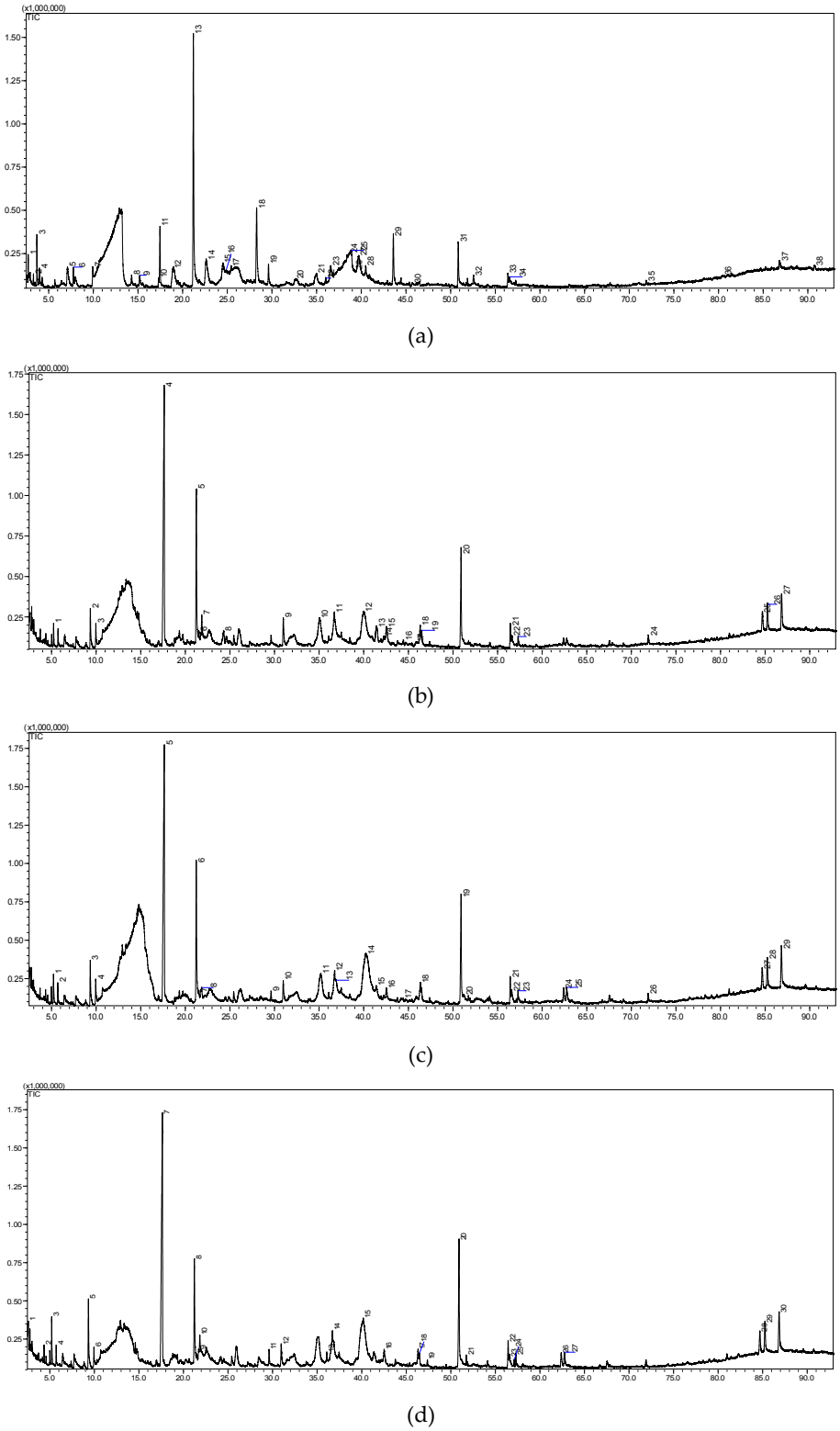

Figure 1: GC-MS chromatogram of ethanolic extract of *Gigantochloa scortechinii* rhizome at Amanjaya Forest Reserve showed peaks of the test compound vs retention time in minutes; (a) new sprout (age-1), (b) young (age-2), (c) pre-mature (age-3), and (d) mature (age-4) rhizome

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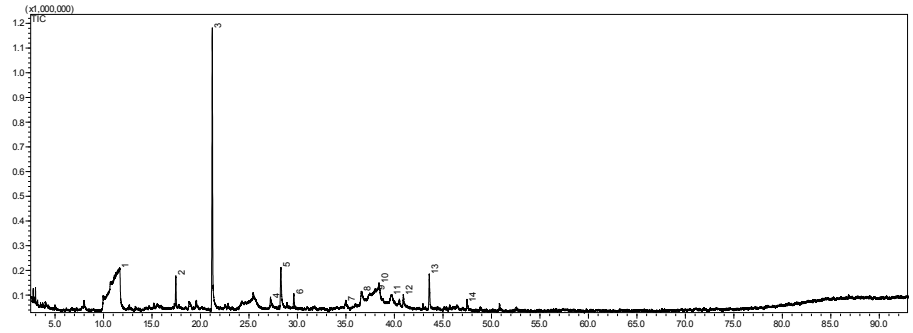
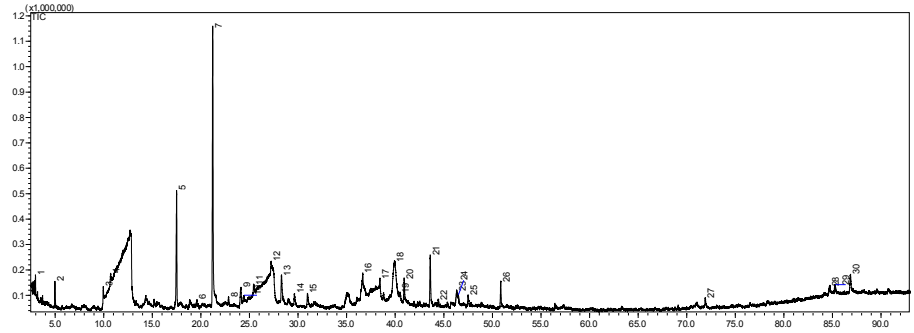


(a)



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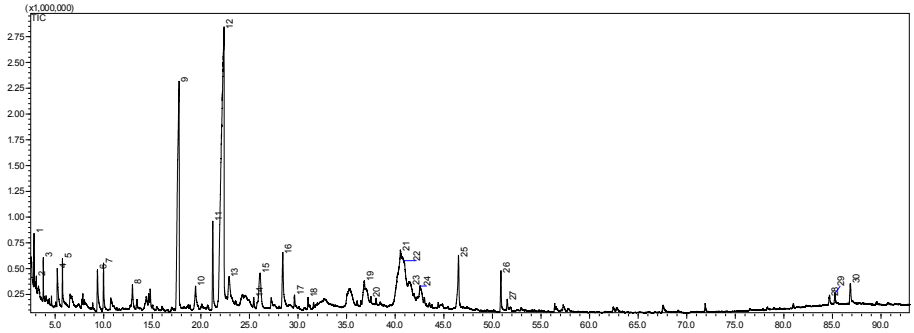

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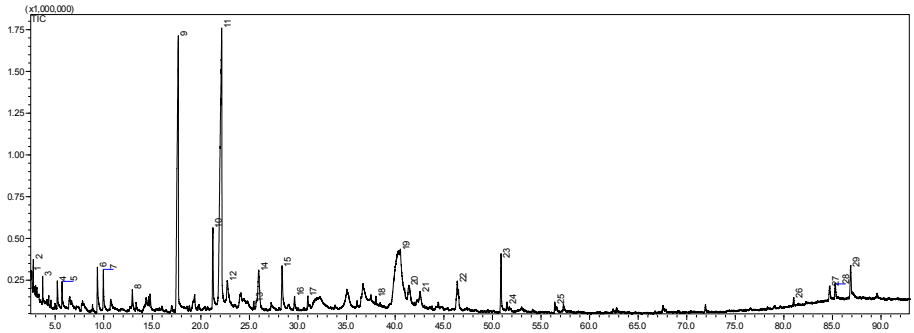

(d)

153 Figure 2: GC-MS chromatogram of ethanolic extract of *Gigantochloa scortechinii* rhizome at Kenaboi
154 Forest Reserve showed peaks of the test compound vs retention time in minutes; (a) new sprout
155 (age-1), (b) young (age-2), (c) pre-mature (age-3), and (d) mature (age-4) rhizome
156

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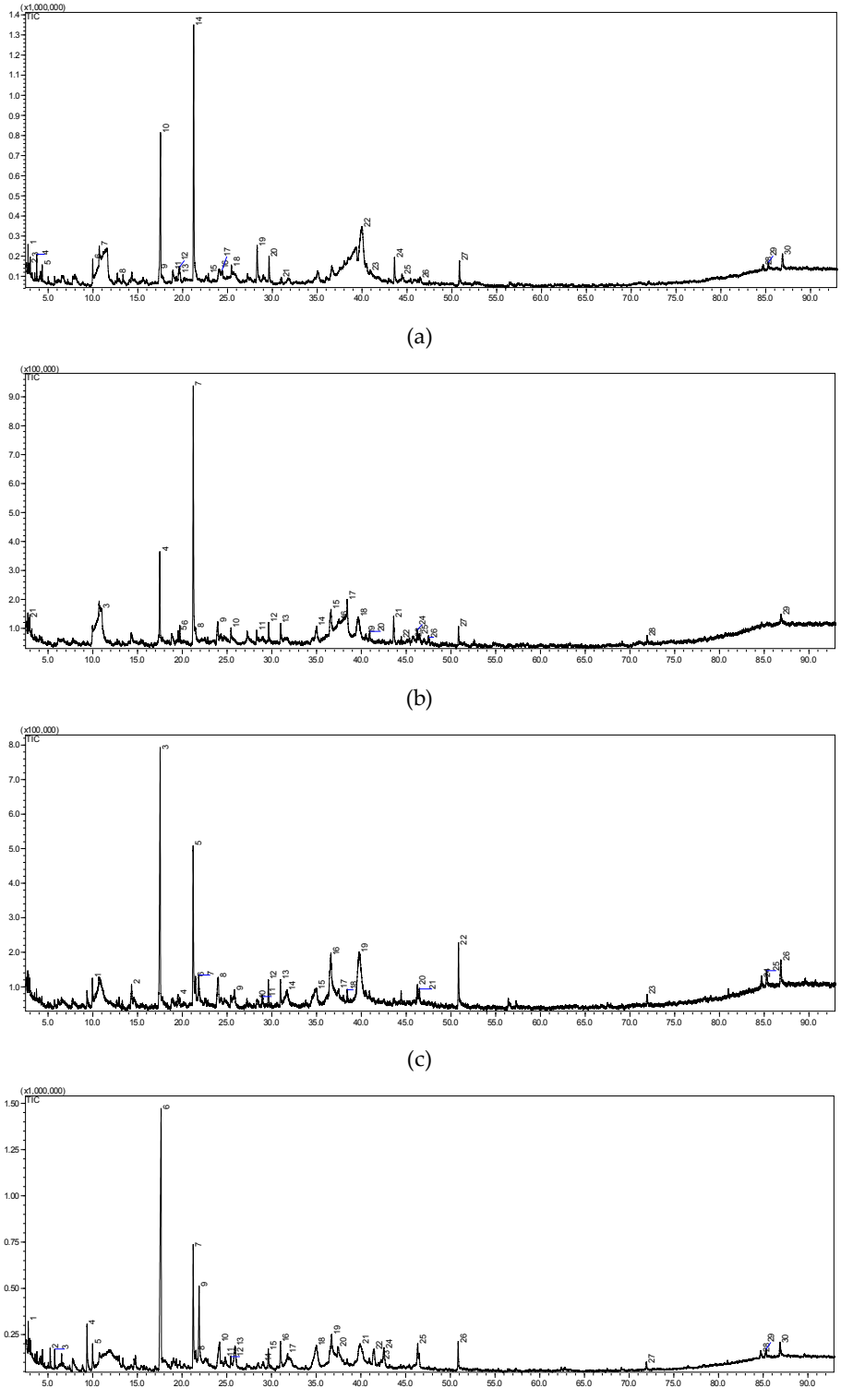

158 Figure 3: GC-MS chromatogram of ethanolic extract of *Gigantochloa scortechinii* rhizome at Ayer
159 Hitam Forest Reserve showed peaks of the test compound vs retention time in minutes; (a) new
160 sprout (age-1), (b) young (age-2), (c) pre-mature (age-3), and (d) mature (age-4) rhizome
161

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162 Table 1: Identification details of phytochemical compounds

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	2-Propanone, 1-Hydroxy- (CAS) 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	$C_{12}H_{24}O_2$	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

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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

163 Table 2: Concentration of phytochemical compounds of four rhizome ages at Amanjaya Forest
164 Reserve

No.	Retention time (min)	Compound	Concentration (%)			
			1	2	3	4
1	2.59±0.00	Butanal, 2-Methyl-	nd	nd	nd	0.56
2	2.71±0.00	2-Propanone, 1-Hydroxy-	0.57	nd	nd	nd
5	3.29±0.00	Glycerin	0.48	nd	nd	nd
6	3.68±0.00	2-Propyn-1-Ol (CAS) Propargyl Alcohol	2.19	nd	nd	nd
7	3.96±0.00	2-Propanone, 1-Hydroxy- (CAS) Acetol	1.35	nd	nd	nd
8	4.36±0.00	2,3-Butanediol, [R-(R*,R*)]-	nd	nd	nd	0.49
10	5.22±0.01	Furfural	nd	1.01	1.37	1.91
11	5.71±0.01	2-Furanmethanol (CAS) Furfuryl Alcohol	nd	nd	0.84	1.08
12	7.76±0.00	Isoamylbutyrate	1.26	nd	nd	nd
13	9.34±0.01	2-Furancarboxaldehyde, 5-Methyl- (CAS) Furfural <5-Methyl->	nd	2.98	2.94	4.95
14	9.95±0.02	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	1.09	1.30	1.33	0.96
17	14.29±0.00	1,3,5-Triazine-2,4,6-Triamine	0.95	nd	nd	nd
18	17.59±0.05	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	5.10	26.20	24.09	27.22
21	21.22±0.01	Benzofuran, 2,3-Dihydro-	23.09	10.31	9.46	7.64
22	21.84±0.01	Hydroxy Methyl Furfural	nd	1.97	0.71	2.45
23	22.63±0.00	1,2,3-Propanetriol, 1-Acetate	6.82	nd	nd	nd
24	24.29±0.00	Malic Acid	nd	1.79	nd	nd
26	25.42±0.00	2-Methoxy-4-Vinylphenol	0.59	nd	nd	nd

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28	28.29±0.00	Benzaldehyde, 4-Hydroxy- (CAS) P-Hydroxybenzaldehyde	9.04	nd	nd	nd
31	29.61±0.00	Benzaldehyde, 4-Hydroxy-3-Methoxy- (CAS) Vanillin	1.85	nd	0.69	1.17
32	30.98±0.01	Phenol, 3,4-Dimethoxy-	nd	2.54	1.67	1.97
34	35.02±0.04	Salicylic Acid <Para->	2.88	6.56	nd	nd
35	36.05±0.02	Dodecanoic Acid (CAS) Lauric Acid	0.56	nd	nd	0.56
36	36.73±0.01	Benzoic Acid, 4-Hydroxy-3-Methoxy-	nd	nd	3.24	6.76
38	38.84±0.00	L-Arabinitol	2.61	nd	nd	nd
39	40.03±0.33	Quinic Acid	4.23	8.95	17.07	7.44
40	40.50±0.00	Benzaldehyde, 4-Hydroxy-3,5-Dimethoxy- (CAS) Syringaldehyde	0.80	nd	nd	nd
42	43.59±0.00	Coniferyl Alcohol	5.55	nd	nd	nd
43	46.34±0.02	Benzoic Acid, 4-Hydroxy-3,5-Dimethoxy-	nd	1.67	1.65	1.25
44	46.49±0.01	Ethanone, 1-(4-Hydroxy-3,5-Dimethoxyphenyl)-	nd	1.02	nd	0.83
45	47.38±0.00	Pentadecanoic Acid	nd	nd	nd	0.33
47	50.90±0.04	N-Hexadecanoic Acid	4.03	8.05	7.81	11.52
48	51.74±0.00	Hexadecanoic Acid, Ethyl Ester (CAS) Ethyl Palmitate	nd	nd	nd	0.59
49	56.43±0.01	9,12-Octadecadienoic Acid (Z,Z)-	1.15	1.72	1.57	1.91
50	56.59±0.00	Hexadec-(9Z)-Enal	1.23	nd	nd	nd
51	56.58±0.00	Cis-Vaccenic Acid	nd	nd	1.45	nd
52	56.60±0.00	7-Tetradecenal, (Z)-	nd	1.40	nd	nd
53	57.11±0.00	Linoleic Acid Ethyl Ester	nd	nd	nd	0.36
54	57.30±0.01	Octadecanoic Acid	nd	0.79	0.92	0.93
55	85.26±0.00	Stigmasterol	nd	2.52	nd	3.08
56	86.84±0.01	Stigmast-5-En-3-Ol, (3.Beta.)- (CAS) 24.Beta.-Ethyl-5.Delta.-Cholesten-3.Beta.-Ol	nd	3.80	3.90	nd

Note: nd = not detected

165 Table 3: Concentration of phytochemical compounds of four rhizome ages at Kenaboi Forest
166 Reserve

No.	Retention time (min)	Compound	Concentration (%)			
			1	2	3	4
1	2.59±0.00	Butanal, 2-Methyl-	nd	nd	nd	0.13
2	2.79±0.04	2-Propanone, 1-Hydroxy-	nd	nd	0.50	0.33
4	3.01±0.04	Ethylene Glycol P5	nd	0.72	0.19	nd
6	3.75±0.03	2-Propyn-1-Ol (CAS) Propargyl Alcohol	nd	nd	0.80	0.44
9	4.98±0.00	Urethane	nd	1.04	nd	nd
10	5.22±0.00	Furfural	nd	nd	2.11	1.25
11	5.73±0.02	2-Furanmethanol (CAS) Furfuryl Alcohol	nd	nd	1.51	0.92
13	9.35±0.00	2-Furancarboxaldehyde, 5-Methyl- (CAS) Furfural <5-Methyl->	nd	nd	2.52	2.34
14	9.96±0.01	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	nd	1.46	1.45	1.66
16	12.95±0.00	Pentanoic Acid, 4-Oxo-	nd	nd	nd	1.18

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18	17.59±0.13	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	3.34	9.70	20.64	19.81
19	19.45±0.00	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	nd	nd	1.69	nd
20	19.59±0.00	5-Methoxypyrrolidin-2-One	nd	0.75	nd	nd
21	21.22±0.01	Benzofuran, 2,3-Dihydro-	32.04	24.67	3.59	3.53
22	22.25±0.11	Hydroxy Methyl Furfural	nd	nd	32.63	27.05
23	22.82±0.09	1,2,3-Propanetriol, 1-Acetate	nd	nd	2.23	1.91
24	24.12±0.00	Malic Acid	nd	2.27	nd	nd
26	25.43±0.00	2-Methoxy-4-Vinylphenol	nd	0.96	0.25	0.26
27	27.23±0.01	Phenol, 2,6-Dimethoxy- (CAS) 2,6-Dimethoxyphenol	1.47	9.92	nd	nd
28	28.34±0.05	Benzaldehyde, 4-Hydroxy- (CAS) P-Hydroxybenzaldehyde	7.27	3.24	3.91	2.78
31	29.64±0.01	Benzaldehyde, 4-Hydroxy-3-Methoxy- (CAS) Vanillin	1.64	1.51	0.60	0.61
32	31.00±0.03	Phenol, 3,4-Dimethoxy-	nd	1.91	0.55	0.69
36	36.74±0.09	Benzoic Acid, 4-Hydroxy-3-Methoxy-	nd	5.22	2.22	nd
37	38.42±0.00	Phenol, 3,4,5-trimethoxy-	nd	1.01	nd	nd
39	40.42±0.49	Quinic Acid	nd	12.07	2.06	18.41
40	40.54±0.00	Benzaldehyde, 4-Hydroxy-3,5-Dimethoxy- (CAS) Syringaldehyde	nd	nd	3.95	nd
41	40.92±0.00	Ethyl 4-Hydroxy-Dl-Mandelate	1.79	2.56	nd	nd
42	43.60±0.00	Coniferyl Alcohol	4.83	4.85	nd	nd
43	46.41±0.10	Benzoic Acid, 4-Hydroxy-3,5-Dimethoxy-	nd	2.01	3.64	2.01
46	47.49±0.01	Ethyl Ester Vanillylmandelic Acid .BETA.-O-Ethyl Ether	1.38	1.28	nd	nd
47	50.88±0.02	N-Hexadecanoic Acid	nd	2.32	1.90	2.70
49	56.43±0.00	9,12-Octadecadienoic Acid (Z,Z)-	nd	nd	nd	0.45
56	86.86±0.01	Stigmast-5-En-3-Ol, (3.Beta.)- (CAS) 24.Beta.-Ethyl-5.Delta.-Cholesten-3.Beta.-Ol	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

No.	Retention time (min)	Compound	Concentration (%)			
			1	2	3	4
2	2.77±0.03	2-Propanone, 1-Hydroxy-	0.70	0.72	nd	0.60
3	2.89±0.01	2-Propenoic acid (CAS) Acrylic acid	0.30	1.07	nd	nd
4	3.00±0.00	Ethylene Glycol P5	0.68	nd	nd	nd
6	3.72±0.00	2-Propyn-1-Ol (CAS) Propargyl Alcohol	0.82	nd	nd	nd
8	4.34±0.00	2,3-Butanediol, [R-(R*,R*)]-	0.70	nd	nd	nd
10	5.24±0.00	Furfural	nd	nd	nd	1.20
11	5.75±0.00	2-Furanmethanol (CAS) Furfuryl Alcohol	nd	nd	nd	0.92
13	9.37±0.00	2-Furancarboxaldehyde, 5-Methyl- (CAS) Furfural <5-Methyl->	nd	nd	nd	3.90

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14	9.97±0.02	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	1.42	nd	1.88	1.74
15	12.72±0.00	N,N'-Dimethylpiperazine	0.82	nd	nd	nd
18	17.54±0.07	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	15.89	10.59	23.77	29.30
20	19.57±0.06	5-Methoxypyrrolidin-2-One	2.79	nd	1.27	nd
21	21.22±0.02	Benzofuran, 2,3-Dihydro-	22.39	28.54	13.02	9.28
22	21.87±0.04	Hydroxy Methyl Furfural	nd	nd	2.56	7.21
24	24.06±0.13	Malic Acid	2.14	4.06	4.69	4.87
25	24.82±0.00	Butanedioic Acid, Hydroxy-, Diethyl Ester, (.+/-.)-	nd	nd	nd	1.36
26	25.44±0.01	2-Methoxy-4-Vinylphenol	1.04	1.60	nd	0.79
28	28.31±0.02	Benzaldehyde, 4-Hydroxy- (CAS) P-Hydroxybenzaldehyde	4.13	1.72	0.23	nd
29	28.94±0.00	Formyl Glutamine	nd	nd	1.29	nd
30	29.07±0.00	L-Proline, 5-Oxo-, Methyl Ester	nd	nd	nd	0.66
31	29.63±0.02	Benzaldehyde, 4-Hydroxy-3-Methoxy- (CAS) Vanillin	2.26	2.13	2.13	1.71
32	30.98±0.03	Phenol, 3,4-Dimethoxy-	nd	2.51	2.17	2.56
33	34.98±0.00	Benzoic acid, 4-hydroxy-, propyl ester (CAS) N-Propyl P-Hydroxybenzoate	nd	nd	0.97	nd
34	34.99±0.00	Salicylic Acid <Para->	nd	3.39	nd	nd
37	38.42±0.00	Phenol, 3,4,5-trimethoxy-	nd	nd	1.02	nd
39	39.83±0.16	Quinic Acid	14.15	8.48	8.86	5.29
42	43.61±0.00	Coniferyl Alcohol	2.66	3.58	nd	nd
43	46.29±0.04	Benzoic Acid, 4-Hydroxy-3,5-Dimethoxy-	nd	1.58	1.76	1.53
44	46.48±0.00	Ethanone, 1-(4-Hydroxy-3,5-Dimethoxyphenyl)-	nd	nd	1.54	nd
47	50.86±0.01	N-Hexadecanoic Acid	2.68	2.28	5.26	2.29

Note: nd = not detected

169 Table 5: Statistical significances of the responses of phytochemical compounds to study site

No.	Compound	Concentration (%)			P-value and statistical significance
		Amanjaya FR	Kenaboi FR	Ayer Hitam FR	
1	Butanal, 2-Methyl-	0.56 ± nc	0.13 ±nc	nd	0.355nsb
2	2-Propanone, 1-Hydroxy-	0.57 ± nc	0.42 ±0.09	0.67 ±0.04	0.104nsa
3	2-Propenoic acid (CAS) Acrylic acid	nd	nd	0.69 ±0.39	nc
4	Ethylene Glycol P5	nd	0.46 ±0.27	0.68 ± nc	0.710nsa
5	Glycerin	0.48 ±nc	nd	nd	nc
6	2-Propyn-1-Ol (CAS) Propargyl Alcohol	2.19	0.62	0.82	0.190nsa

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		±nc	±0.18	±nc	
7	2-Propanone, 1-Hydroxy- (CAS) Acetol	1.35 ±nc	nd	nd	nc
8	2,3-Butanediol, [R-(R*,R*)]-	0.49 ±nc	nd	0.70 ±nc	0.111nsb
9	Urethane	nd	1.04 ±nc	nd	nc
10	Furfural	1.43 ±0.26	1.68 ±0.43	1.20 ±nc	0.749nsa
11	2-Furanmethanol (CAS) Furfuryl Alcohol	0.96 ±0.12	1.22 ±0.30	0.92 ±nc	0.699nsa
12	Isoamylbutyrate	1.26 ±nc	nd	nd	nc
13	2-Furancarboxaldehyde, 5-Methyl- (CAS) Furfural <5-Methyl->	3.62 ±0.66	2.43 ±0.09	3.90 ±nc	0.407nsa
14	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	1.17 ±0.09	1.52 ±0.07	1.68 ±0.14	0.019*a
15	N,N'-Dimethylpiperazine	nd	nd	0.82 ±nc	nc
16	Pentanoic Acid, 4-Oxo-	nd	1.18 ±nc	nd	nc
17	1,3,5-Triazine-2,4,6-Triamine	0.95 ±nc	nd	nd	nc
18	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	20.65 ±5.22	13.37 ±4.17	19.89 ±4.14	0.489nsa
19	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	nd	1.69 ±nc	nd	nc
20	5-Methoxypyrrolidin-2-One	nd	0.75 ±nc	2.03 ±0.76	0.509nsa
21	Benzofuran, 2,3-Dihydro-	12.63 ±3.53	15.96 ±7.31	18.31 ±4.39	0.757nsa
22	Hydroxy Methyl Furfural	1.71 ±0.52	29.84 ±2.79	4.89 ±2.33	0.001**a
23	1,2,3-Propanetriol, 1-Acetate	6.82 ±nc	2.07 ±0.16	nd	0.037*a
24	Malic Acid	1.79 ±nc	2.27 ±nc	3.94 ±0.62	0.337nsa
25	Butanedioic Acid, Hydroxy-, Diethyl Ester, (.+/-.)-	nd	nd	1.36±nc	nc
26	2-Methoxy-4-Vinylphenol	0.59 ±nc	0.49 ±0.24	1.14 ±0.24	0.247nsa
27	Phenol, 2,6-Dimethoxy- (CAS) 2,6-Dimethoxyphenol	nd	5.70 ±4.23	nd	nc
28	Benzaldehyde, 4-Hydroxy- (CAS)	9.04	4.30	2.03	0.072nsa

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	P-Hydroxybenzaldehyde	±nc	±1.02	±1.14	
29	Formyl Glutamine	nd	nd	1.29 ±nc	nc
30	L-Proline, 5-Oxo-, Methyl Ester	nd	nd	0.66 ±nc	nc
31	Benzaldehyde, 4-Hydroxy-3-Methoxy- (CAS) Vanillin	1.24 ±0.34	1.09 ±0.28	2.06 ±0.12	0.045*a
32	Phenol, 3,4-Dimethoxy-	2.06 ±0.26	1.05 ±0.43	2.41 ±0.12	0.042*a
33	Benzoic acid, 4-Hydroxy-, Propyl Ester (CAS) N-Propyl P-Hydroxybenzoate	nd	nd	0.97 ±nc	nc
34	Salicylic Acid <Para->	4.72 ±1.84	nd	3.39 ±nc	0.748nsa
35	Dodecanoic Acid (CAS) Lauric Acid	0.56 ±0.00	nd	nd	nc
36	Benzoic Acid, 4-Hydroxy-3-Methoxy-	5.00 ±1.76	3.72 ±1.50	nd	0.636nsa
37	Phenol, 3,4,5-Trimethoxy-	nd	1.01 ±nc	1.02 ±nc	0.003**b
38	L-Arabinitol	2.61±nc	nd	nd	nc
39	Quinic Acid	9.42 ±2.73	10.85 ±4.76	9.20 ±1.84	0.925nsa
43	Benzoic Acid, 4-Hydroxy-3,5-Dimethoxy-	1.52 ±0.14	2.55 ±0.54	1.62 ±0.07	0.123nsa
44	Ethanone, 1-(4-Hydroxy-3,5-Dimethoxyphenyl)-	0.93 ±0.10	nd	1.54 ±nc	0.166nsa
45	Pentadecanoic Acid	0.33 ±nc	nd	nd	nc
46	Ethyl Ester Vanillylmandelic Acid .BETA.-O-Ethyl Ether	nd	1.33 ±0.05	nd	nc
47	N-Hexadecanoic Acid	7.85 ±1.53	2.32 ±0.23	3.13 ±0.72	0.014*a
48	Hexadecanoic Acid, Ethyl Ester (CAS) Ethyl Palmitate	0.59 ±nc	nd	nd	nc
49	9,12-Octadecadienoic Acid (Z,Z)-	1.59 ±0.16	0.45 ±nc	nd	0.051nsa
50	Hexadec-(9Z)-Enal	1.23 ±nc	nd	nd	nc
51	Cis-Vaccenic Acid	1.45 ±nc	nd	nd	nc
52	7-Tetradecenal, (Z)-	1.40 ±nc	nd	nd	nc
53	Linoleic Acid Ethyl Ester	0.36	nd	nd	nc

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		±nc			
54	Octadecanoic Acid	0.88 ±0.05	nd	nd	nc
55	Stigmasterol	2.80 ±0.28	nd	nd	nc
56	Stigmast-5-En-3-Ol, (3.Beta.)- (Cas) 24.Beta.-Ethyl-5.Delta.-Cholesten-3.Beta.-Ol	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

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171 Table 6: Statistical significances of the responses of phytochemical compounds to rhizome ages

No.	Compound	Concentration (%)				P-value and statistical significance
		1	2	3	4	
1	Butanal, 2-Methyl-	Nd	nd	nd	0.35 ±0.22	nc
2	2-Propanone, 1-Hydroxy-	0.64 ±0.07	0.72 ±nc	0.50 ±nc	0.47 ±0.14	0.577nsa
3	2-Propenoic acid (CAS) Acrylic acid	0.30 ±nc	1.07 ±nc	nd	nd	0.326nsb
4	Ethylene Glycol P5	0.68 ±nc	0.72 ±nc	0.19 ±nc	nd	0.09nsb
5	Glycerin	0.48 ±nc	nd	nd	nd	nc
6	2-Propyn-1-Ol (CAS) Propargyl Alcohol	1.51 ±0.69	nd	0.80 ±nc	0.44 ±nc	0.725nsa
7	2-Propanone, 1-Hydroxy- (CAS) Acetol	1.35 ±nc	nd	nd	nd	nc
8	2,3-Butanediol, [R-(R*,R*)]-	0.70 ±nc	nd	nd	0.49 ±nc	0.111nsb
9	Urethane	Nd	1.04 ±nc	nd	nd	nc
10	Furfural	Nd	1.01 ±nc	1.74 ±0.37	1.45 ±0.23	0.490nsa
11	2-Furanmethanol (CAS) Furfuryl Alcohol	Nd	nd	1.18 ±0.34	0.97 ±0.05	0.493nsa
12	Isoamylbutyrate	1.26 ±nc	nd	nd	nd	nc
13	2-Furancarboxaldehyde, 5-Methyl- (CAS) Furfural <5-Methyl->	Nd	2.98 ±nc	2.73 ±0.21	3.73 ±0.76	0.625nsa
14	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	1.26 ±0.17	1.38 ±0.08	1.55 ±0.17	1.45 ±0.25	0.774nsa

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15	N,N'-Dimethylpiperazine	0.82 ±nc	nd	nd	nd	nc
16	Pentanoic Acid, 4-Oxo-	Nd	nd	nd	1.18 ±nc	nc
17	1,3,5-Triazine-2,4,6-Triamine	0.95 ±nc	nd	nd	nd	nc
18	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	8.11 ±3.92	15.50 ±5.36	22.83 ±1.10	25.44 ±2.88	0.039*a
19	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	Nd	nd	1.69±nc	nd	nc
20	5-Methoxypyrrolidin-2-One	2.79 ±nc	0.75 ±nc	1.27 ±nc	nd	0.120nsb
21	Benzofuran, 2,3-Dihydro-	25.84 ±3.11	21.17 ±5.55	8.69 ±2.75	6.82 ±1.71	0.013*a
22	Hydroxy Methyl Furfural	Nd	1.97 ±nc	11.97 ±10.35	12.24 ±7.53	0.842nsa
23	1,2,3-Propanetriol, 1-Acetate	6.82 ±nc	nd	2.23 ±nc	1.91 ±nc	0.148nsb
24	Malic Acid	2.14 ±nc	2.71 ±0.69	4.69 ±nc	4.87 ±nc	0.410nsa
25	Butanedioic Acid, Hydroxy-, Diethyl Ester, (.+/-.)-	Nd	nd	nd	1.36 ±nc	nc
26	2-Methoxy-4-Vinylphenol	0.82 ±0.23	1.28 ±0.32	0.25 ±nc	0.53 ±0.27	0.286nsa
27	Phenol, 2,6-Dimethoxy- (CAS) 2,6-Dimethoxyphenol	1.47 ±nc	9.92 ±nc	nd	nd	0.406nsb
28	Benzaldehyde, 4-Hydroxy- (CAS) P-Hydroxybenzaldehyde	6.81 ±1.44	2.48 ±0.76	2.07 ±1.84	2.78 ±nc	0.203nsa
29	Formyl Glutamine	Nd	nd	1.29 ±nc	nd	nc
30	L-Proline, 5-Oxo-, Methyl Ester	Nd	nd	nd	0.66 ±nc	nc
31	Benzaldehyde, 4-Hydroxy-3-Methoxy- (CAS) Vanillin	1.92 ±0.18	1.82 ±0.31	1.14 ±0.50	1.16 ±0.32	0.328nsa
32	Phenol, 3,4-Dimethoxy-	Nd	2.32 ±0.21	1.46 ±0.48	1.74 ±0.55	0.423nsa
33	Benzoic acid, 4-Hydroxy-, Propyl Ester (CAS) N-Propyl P-Hydroxybenzoate	Nd	nd	0.97 ±nc	nd	nc
34	Salicylic Acid <Para->	2.88 ±nc	4.98 ±1.59	nd	nd	0.585nsa
35	Dodecanoic Acid (CAS) Lauric Acid	0.56 ±nc	nd	nd	0.56 ±nc	nc

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36	Benzoic Acid, 4-Hydroxy-3-Methoxy-	Nd	5.22 ±nc	2.73 ±0.51	6.76 ±nc	0.205nsa
37	Phenol, 3,4,5-Trimethoxy-	Nd	1.01 ±nc	1.02 ±nc	nd	0.003**b
38	L-Arabinitol	2.61 ±nc	nd	nd	nd	nc
39	Quinic Acid	9.19 ±4.96	9.83 ±1.13	9.33 ±4.34	10.38 ±4.06	0.996nsa
40	Benzaldehyde, 4-Hydroxy-3,5-Dimethoxy- (CAS) Syringaldehyde	0.80 ±nc	nd	3.95 ±nc	nd	0.373nsb
41	Ethyl 4-Hydroxy-Dl-Mandelate	1.79 ±nc	2.56 ±nc	nd	nd	0.112nsb
42	Coniferyl Alcohol	4.35 ±0.87	4.22 ±0.64	nd	nd	0.921nsa
43	Benzoic Acid, 4-Hydroxy-3,5-Dimethoxy-	Nd	1.75 ±0.13	2.35 ±0.65	1.60 ±0.22	0.428nsa
44	Ethanone, 1-(4-Hydroxy-3,5-Dimethoxyphenyl)-	Nd	1.02 ±nc	1.54 ±nc	0.83 ±nc	0.034*b
45	Pentadecanoic Acid	Nd	nd	nd	0.33 ±nc	nc
46	Ethyl Ester Vanillylmandelic Acid .BETA.-O-Ethyl Ether	1.38 ±nc	1.28 ±nc	nd	nd	0.024*b
47	N-Hexadecanoic Acid	3.36 ±0.68	4.22 ±1.92	4.99 ±1.71	5.50 ±3.01	0.921nsa
48	Hexadecanoic Acid, Ethyl Ester (CAS) Ethyl Palmitate	Nd	nd	nd	0.59 ±nc	nc
49	9,12-Octadecadienoic Acid (Z,Z)-	1.15 ±nc	1.72 ±nc	1.57 ±nc	1.18 ±0.73	0.956nsa
50	Hexadec-(9Z)-Enal	1.23 ±nc	nd	nd	nd	nc
51	Cis-Vaccenic Acid	Nd	nd	1.45 ±nc	nd	nc
52	7-Tetradecenal, (Z)-	Nd	1.40 ±nc	nd	nd	nc
53	Linoleic Acid Ethyl Ester	Nd	nd	nd	0.36 ±nc	nc
54	Octadecanoic Acid	Nd	0.79 ±nc	0.92 ±nc	0.93 ±nc	0.003**b
55	Stigmasterol	Nd	2.52 ±nc	nd	3.08 ±nc	0.063nsb
56	Stigmast-5-En-3-Ol, (3.Beta.)- (Cas)	Nd	3.80	2.58	2.00	0.818nsa

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24.Beta.-Ethyl-5.Delta.-Cholesten-3.Beta.-Ol	±nc	±1.32	±nc

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

Result (Table 6) also indicates that the pattern of the composition with the changes of rhizome age, such as some of the identified compounds present in new sprout and young rhizome but not present after (for example; 2-propenoic acid (CAS) acrylic acid (Rt: 2.89±0.01), phenol, 2,6-dimethoxy- (CAS) 2,6-dimethoxyphenol (Rt: 27.23±0.01), and salicylic acid <para-> (Rt: 35.01±0.02), and some compound present from young to mature rhizome but not present before (for example; furfural (Rt: 5.22±0.02), 2-furancarboxaldehyde,5-methyl- (CAS) furfural <5-methyl-> (Rt: 9.34±0.01), and phenol, 3,4-dimethoxy- (Rt: 9.35±0.02). The irregular pattern of the compound composition were also observed such as for 2-propyn-1-ol (CAS) propargyl alcohol (Rt: 3.72±0.00), 2,3-butanediol,[r-(r*,r*)]- (Rt: 4.35±0.01), 1,2,3-propanetriol,1-acetate (Rt: 22.75±0.03), and dodecanoic acid (CAS) lauric acid (Rt: 36.05±0.02) which present at younger and older-age rhizome but not present at middle-age rhizome.

From 56 identified compounds, results in Table 6 indicates that only 11 compounds present were similar at all four rhizome ages. The concentration of both 4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl and benzofuran, 2,3-dihydro- were found significantly different (p<0.05) with rhizome age. The 4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl was found increased with increasing of rhizome age (8.11±3.92, 15.50±5.36, 22.83±1.10, and 25.44±2.88 % respectively) while the benzofuran, 2,3-dihydro- 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 similar compounds (2-propanone,1-hydroxy-, 2,4-dihydroxy-2,5-dimethyl-3(2h)-furan-3-one, malic acid, 2-methoxy-4-vinylphenol, benzaldehyde, 4-hydroxy- (CAS) p-hydroxybenzaldehyde, benzaldehyde, 4-hydroxy-3-methoxy- (CAS) vanillin, quinic acid, n-hexadecanoic acid, and 9,12-octadecadienoic acid (z,z)- were found not significantly different with rhizome age at p<0.05.

Regarding study site and rhizome age, although great dissimilarity of compound composition and concentration has been observed such as for butanal, 2-methyl-, glycerin, 2,3-butanediol, [r-(r*,r*)]-, stigmasterol, and others, similar compound composition at all three study sites and four rhizome ages has been observed. Both 4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl and benzofuran, 2,3-dihydro- were present at all study sites and rhizome ages (Table 2, 3, and 4).

4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP) (Rt: 17.57±0.17) was found higher at Amanjaya FR (20.65±5.22 %) followed by at Ayer Hitam FR (19.89±4.14 %) and Kenaboi FR (13.37±4.17 %)(Table 5), and higher at the 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, allowed saponins to scavenge superoxides by forming hydroperoxide intermediates and preventing bio-molecular damage by free radicals. DDMP also revealed a strong antioxidant through 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging activity [44]. Higher fungi such as *Schizophyllum commune* is one of the common major sources for this compound [45]. GC-MS analysis of methanolic extract of medicinal herb (*Clerodendrum viscosum*) leaves (traditional used in Indian medicine for asthma, fever, bronchitis, skin diseases, epilepsy, inflammation, tumors, worm infestation and snake bite) also present DDMPP (4.19 %) as one of major compound detected [46], and only 4.0 % in methanolic extract of *Nerium oleander* stems [47], however, the concentration remained lower compared with DDMP present in the ethanolic extract of mature *G. scortechinii*'s rhizome (19.81 – 29.30 %).

Benzofuran, 2,3-dihydro- (RT: 21.22±0.02), 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), and higher at the new sprout (25.84±3.11 %) followed by young (21.17±5.55 %),

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premature (8.69±2.75 %), and mature rhizome (6.82±1.71 %)(Table 5). Coumaran and its derivatives was patented in 1989 [48] (see United States Patent, Patent number 4,857,516) and these compound found potent pharmaceutical activities, such as improving cardiovascular system and anti-allergic activities, e.g. scavenging action of active oxygen species, inhibition of thromboxane A2 synthetase (thromboxane A2 exerts coagulation of blood platelet as well as contraction of blood vessels) or inhibition of 5-lipoxygenase which is a key enzyme for the biosynthesis of leukotrienes. Derivatives of coumaran for example, 3-(Benzofuran-2-yl)-4,5-dihydro-5- arylpyrazole-1-carbothioamide and 3-(benzofuran-2-yl)-4,5-dihydro-5-aryl-1-[4- (aryl)-1,3-thiazol-2-yl]-1H-pyrazoles shows antibacterial and antifungal activities, triazolo[1,5-a]-3-N-alkyl/aryl-4-oxopyrimido [5,4-b] benzofurans (31) and 3,4-dihydro-3- N-alkyl/aryl-4-oxopbenzofuro-[3,2-d] pyrimidine-2-thioacetic acids are potent to exhibit *Staphylococcus aureus* and *Escherichia coli* activity [49]. The concentration of coumaran present in new sprout *G. scortechinii* rhizome (22.39 – 32.04 %) in present study was significantly higher than that found in the methanol extract of Nerium orleander (traditionally used for various illness in China and India) leaves (13.73 %) and stems (4.0 %) in previous study which showed potent bioactivities such as antioxidant, hepatoprotective, analgesic, anti-ulcer, anticancer immunomodulatory and antidiabetic activities [47].

The similar compound composition which is present in all three older-ages rhizomes (from young to mature rhizome) but not present at the new sprout were also observed for the phenol,3,4-dimethoxy- (Rt: 30.99±0.02) and benzoic acid,4-hydroxy-3,5-dimethoxy- (Rt: 46.35±0.01) compound at all three study sites (Table 2, 3, and 4). The concentration of phenol,3,4-dimethoxy- was found significantly different ($p<0.05$) while benzoic acid,4-hydroxy-3,5-dimethoxy- was found not significantly different with study site at $p<0.05$ (Table 5). Both compounds were found not significantly different in concentration between rhizome ages (from young to mature rhizome) at $p<0.05$ (Table 6).

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 [50]; a phenolic compound with yellow crystalline needle structure; which found most potent antioxidant among phenolic compounds [51], and potent adult beetles (*Acalymma vitatum*) antifeedant with up to 100 % feeding inhibition [52]. Phenolic compounds are important organic synthetic and compounds to produce synthetic fibers, fuels, and pesticides, as by-products. Phenol, 3,4-dimethoxy- was reported found in the extracts of bacterial fermentation broth of *Anguilla japonica*, *Sphyraena japonica*, and *Engraulis japonicus* [51], and in tobacco smoke, however did not present in the tobacco itself [53]. In present study, results showed that phenol, 3,4-dimethoxy- concentration was 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, but lower compared to Poplar lignin (4.72 %) [54].

Benzoic acid, 4-hydroxy-3,5-dimethoxy- (HDMBA) found a minor compound in the ethanolic extract at all three study sites (1.25 – 3.64 %). HDMBA or syringic acid is one of the hydroxyl benzoic acid derivatives which are a phenolic compound with colorless crystallized needle structure [55]. Syringic acid has potential beneficial as an antioxidant [56]. This compound found in beer [57], honey [58], tobacco and tobacco smoke [53]. It also found in lees of bokbunja wine (LBW) made from *Rubus coreanus* and showed strong anti-coagulation and platelet aggregation inhibitory activities without hemolytic effect against human blood cells [59]. Syringic acid isolated from truffle *Elaphomyces granulatus* showed strong inhibition of cyclooxygenase-2 (COX-2) enzyme activity (IC_{50} of 0.4 ug/ml) (which resulted to potent anti-inflammation, aging, cancer and heart disease), and strong antioxidant activity (IC_{50} of 0.7 µg/ml) with no cytotoxic effect on myelomonocytic HL-60 cells (ATCC) (up to 31.25 µg/ml concentration of syringic acid) [60]. Syringic acid found in *Eugenia polyantha* leaf (traditional use to treat diabetic and as food additives) showed a higher level of inhibition of alpha-glucosidases activity (AG) (35 %) compare to other hydroxyl benzoic acid derivatives such as 4-hydroxy-3-methoxy benzoic acid (25 %) and 3,4,5-trihydroxy benzoic acid (20 %). HDMBA showed free radical scavenging activity (DPPH) (89 %) and beta-carotene bleaching protection (BC) (44 %) [61].

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Furthermore, quinic acid (Rt: 40.06±0.15) and n-hexadecanoic acid (Rt: 50.88±0.00) compounds were present in all three study sites and all four rhizome ages except for new sprout at Kenaboi FR (Table 2, 3 and 4). The quinic acid was found not significantly different while n-hexadecanoic acid was found significantly 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 quinic and n-hexadecanoic acid were found not significantly different with rhizome age at $p<0.05$ (Table 6).

The quinic acid was found from 2.06 to 18.41 % in the ethanolic extract of the rhizome. Quinic acid is a flavonoid compound also known as cyclohexane carboxylic acid; commonly obtained from cinchona bark and coffee beans. [62] demonstrate that quinic acids derivatives, KZ-41 would inhibit glucose-induced apoptosis in retinal endothelial cells (REC) and promote REC survival under various genotoxic stresses from the suffering of diabetic retinopathy. Moreover, application of quinic acid found treated groups down-regulated hyperglycemia and oxidative stress by up-regulating insulin and C-peptide levels. Application bi-flavonoids (quercetin and quinic acid) 50mg/kg exhibited maximum inhibition of the pro-apoptotic protein Bax expression and therefore demonstrate in ameliorating hyperglycemia, hyperlipidemia and insulin resistance in diabetic [63]. A derivative of quinic acid such as caffeoylated quinic acids (CQAs) showed potent to ameliorate high mobility group box 1 protein (HMGB1) - mediated vascular barrier disruption [64]. Quinic acid ester also found higher on antioxidant activity using the DPPH radical scavenging activity assay and for their antiherpes activity against HSV-1 than α-tocopherol [65].

The n-hexadecanoic acid or so-called palmitic acid is a fatty acid (FA) found from 1.90 to 11.52 % in the ethanolic extract of the rhizome. Compared to another finding on traditional medicinal plant, methanol extract of *Nerium oleander*'s leaves showed higher concentration palmitic acid (52 %), lower in n-hexane extract *Nerium oleander*'s stem (3.0 %) [47], an average (5.91 %) in supernatant mycelial extract of the fungus *Aspergillus unguis* and lower (1.11 %) in supernatant static extract of fungus *A. unguis* [66]. N-hexadecanoic acid is a potent inhibitor of phospholipase A_2, hence, as an anti-inflammatory compound and for rheumatic symptoms [67]. Basically, FA is considered as indispensable compounds for the efficiency of medicinal plants, and hence as constitute essential structural elements of biological membranes in all organism [19]. However, increasing concentration of N-hexadecanoic; predominant saturated free fatty acid from adipose tissue, but not unsaturated present in obese and insulin resistance individual induced production of IP-10 by human macrophages [68]. In addition, acute stimulation of palmitic acid and high-fat diets (HFD) resulted to endothelium dependent vasodilatation (EDV) impairment and increased endoplasmic reticulum (ER) stress in dose-time dependent manner [69]. The increment of palmitic acid also induces apoptosis in dose-time dependent manner of neural stem cells (NCSs) which may contribute to a neurological disorder [70].

3. Materials and Methods

3.1 Sampling

Samples of rhizome of *G. scortechinii* were collected at three different locations in Peninsular Malaysia: (1) 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; (2) 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 (3) 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 had 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 sampling was conducted after the rainy season, when the growing season starts and a high number of shoots sprout. Using the selected randomized method, samples were collected from healthy clumps under natural stand conditions at Amanjaya FR and Kenaboi FR, while only planted

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bamboo stands were sampled at Ayer Hitam FR. Clumps that were not fertile or were congested were ignored. In each sample, three clumps were selected and used as three replicates. Four consecutive rhizomes (Figure 4) from each clump—i.e., new sprouts 1.0–2.5 m high (estimated age of < one month), young rhizomes (estimated age of one year), pre-mature rhizomes (estimated age of two years), and mature rhizomes (estimated age of three years)—were selected.

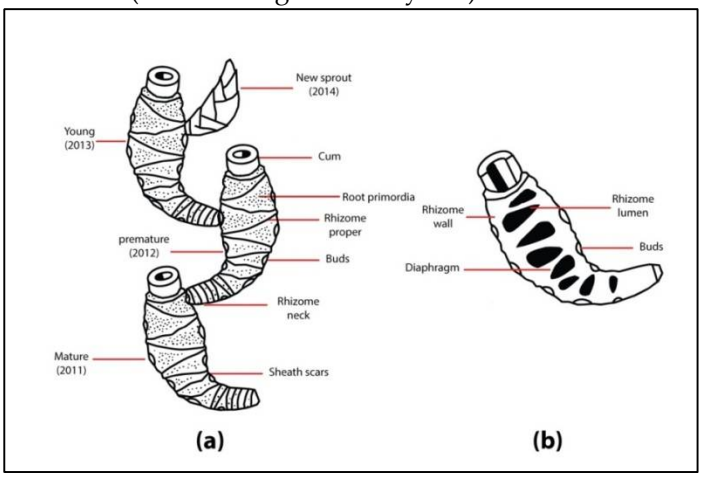

Figure 4: Diagram of (a) four consecutive *Gigantochloa scortechinii* rhizomes, and (b) transverse section of rhizome

3.2 Sample preparation

The 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 such as in wet state sample [71].

3.3 Solvent extraction

20 g of homogenized sieve dried sample (composited from three replicate into one sample) were extracted using 200 ml solvent extraction (70 ethanol: 30 water) with soxhlet apparatus and evaporated using rotary evaporator at 40 °C. [72]. Samples were composited and homogenized. The composited and homogenization procedure was conducted in a very wary mode to ensure a homogenized sample were prepared. Ethanol was selected for this study because it was found to be more efficient in cell wall degradation, preserve several enzymes from degraded and easier to penetrate the cellular membrane compared to water, acetone, chloroform, and ether. Ethanol also much more suitable than methanol due to toxicity level of methanol, and by adding 30 % of water was to increase the polarity of extraction solvent [73,74].

3.4 Gas Chromatography-Mass Spectrometry 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 parameter used: 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. Identification of compound detected was done using 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.

3.5 Statistical analysis

The effect of the different study site and age-related on phytochemical concentration was analyzed using either one-way analysis of variance or the one-sample t-test. Two-way analysis of variance was not used in the analysis due to having no replication of the sample and most of the identified phytochemical composition did not meet the assumption requirement. The statistical analysis was done using the IBM SPSS statistics version 21.0.

4. Conclusions

Both study site and age-related showed a great variation of phytochemical composition and concentration. 18 compounds were found similar at all three study sites, however, their 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 similar compound composition and concentration. However, an analysis with replication is needed to enhance the statistical analysis which further with an accurate figure.

The GC-MS analysis of ethanolic extract of *G. scortechinii* also exposes a large number (56) of phytochemical compounds identified in *G. scortechinii* rhizome which depicted its potential to be exploiting the potent bio-activity. Several beneficial phytochemical compounds such as 4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, benzofuran, 2,3-dihydro-, phenol,3,4-dimethoxy-, benzoic acid,4-hydroxy-3,5-dimethoxy-, quinic acid, and n-hexadecanoic acid were recommended based on their potent functional properties and their availability (similarity 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 improve the understanding on phytochemical compounds in bamboo and hence promote the utilization regarding to plant derive bio-material.

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