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CHEMICAL AND BIOLOGICAL STUDIES OF *ZINGIBER PHILLIPSEAE* RHIZOMES EXTRACT

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ABSTRACT

A novel flavonoidic compound, 3,5-dihydroxy-4',7-dimethoxyflavone, has been isolated from the rhizomes of *Zingiber phillipseae*. The dichloromethane extract of this species was shown to be non-toxic with LC_{50} for acute and toxic levels at 9,732 ppm and 562 ppm, respectively. The extract showed higher antioxidant activity than the standard (butylated hydroxytoluene, BHT) but lower than dichloromethane extract of *Zingiber officinale*. It showed significant activity against *Staphylococcus aureus* and *Escherichia coli* with minimum inhibitory concentration (MIC) at 200µg/ml and 225 µg/ml respectively. Both extracts were found negative toward *Candida albicans*.

KEYWORDS: ***Zingiber phillipseae*; 3,5-dihydroxy-4',7-dimethoxyflavone; brine shrimp test; antioxidant activity; antimicrobial activity**

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INTRODUCTION

Malaysia is endowed with a remarkable biodiversity and many plants with therapeutic properties have yet to be discovered. In addition, the nutritional and health properties of many of these plants remain unknown and the associated scientific research is insufficient, inconsequential and ignoring the great therapeutic potential of these plants.

Existence of such plants, the Zingiberaceae have a pantropical distribution in the tropics of Africa, Asia and the Americas, with their greatest diversity in Southeast Asia. Detailed specifications to the family have been described elsewhere[1]. *Zingiber phillipseae* (ZINGIBERACEAE) is of the same genus as household ginger, *Zingiber officinale* and endemic to the Crocker Range in Sabah. The species is named by Theilade and Mood[2] after Mrs. Susan M. Phillips, a local resident of Sabah who has contributed to public awareness on the conservation of flora and fauna of Borneo through her artwork and writings.

To date, little is known about this plant aside from a taxonomic description[2]. Information on its chemical constituents and biological activities remain unreported. In view of this, we proceeded to screen dichloromethane extracts of *Zingiber phillipseae* rhizomes. The extracts were subjected to brine shrimp toxicity test, antimicrobial and antioxidant activities study. The compound was isolated and structurally elucidated by conventional spectroscopic and chemical techniques.

MATERIALS AND METHODS

General procedures

IR spectra were recorded on Thermo Nicolet model Nexus 670 FT spectrophotometer; ^{1}H ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra were recorded on JEOL ECA operating at 600 MHz and 100 MHz respectively; MS spectra were measured on an LC-MS/MS Thermo Finnigan LCQ spectrometer.

Reagents, chemicals and microorganisms

Reagents were of Analytical or HPLC grade obtained from Fisher USA. Fine chemicals were obtained from Merck. Cysts of *Artemia salina* were obtained from Wudi Shoreline Feed Co. Ltd. The microorganisms, *Escherichia coli* (gram-negative bacteria), *Staphylococcus aureus* (gram-positive bacteria) and *Candida albicans* (fungus) were sourced from The National Microbes Bank, Institute for Medical Research (Kuala Lumpur).

Plant material

Zingiber phillipseae plants were collected in the vicinity of 39 km from Kota Kinabalu-Tambunan Road (Crocker Range, Sabah) during August to November of 2002 with the assistance of Anthony E. Lamb (Manager of the Sabah Agriculture Park, Tenom) and verified by the taxonomist, Ida M. Thelaide. Fresh rhizomes (1.0 kg) were rinsed off to remove debris and cut into small pieces (2 cm).

Preparation of the extracts

The dichloromethane extract of the rhizomes of *Zingiber phillipseae* was prepared by using Soxhlet apparatus. The dichloromethane extracts were then consolidated and concentrated in vacuo to give a yellow brown oily mass (yield 0.11%). The dichloromethane extract was then subjected to phytochemical analysis using standard methods. Similarly, the rhizomes of *Zingiber officinale* were extracted with dichloromethane and reduced in vacuo to give yellowish oil (yield 1.02%).

1. Isolation and Structure Elucidation of Flavone

Extraction and Isolation

The dichloromethane extract was subjected to Waters 600 HPLC and the solvent system used was modified from Hiserodt[3] (Table 1). The HPLC chromatogram is shown in Figure 1. Fractions were collected using the semi-preparative column Xterra Rp18 7μm and Fraction Collector II. Compound corresponding to peak 4 (t_r 15.9 -16.7 min) was concentrated in vacuo to give a yellow residue which was subjected to successive chromatography on silica gel using dichloromethane as solvent followed by recrystallization resulting in yellowish needles (9.64 mg; 0.88%).

Table 1
HPLC gradient system

Time (min)	Rate (min/mL)	acetonitrile (%)	water (%)
0.00	1.00	60.00	40.00
10.00	1.00	50.00	50.00
25.00	1.00	35.00	65.00
26.00	1.00	0.00	100.00
35.00	1.00	60.00	40.00

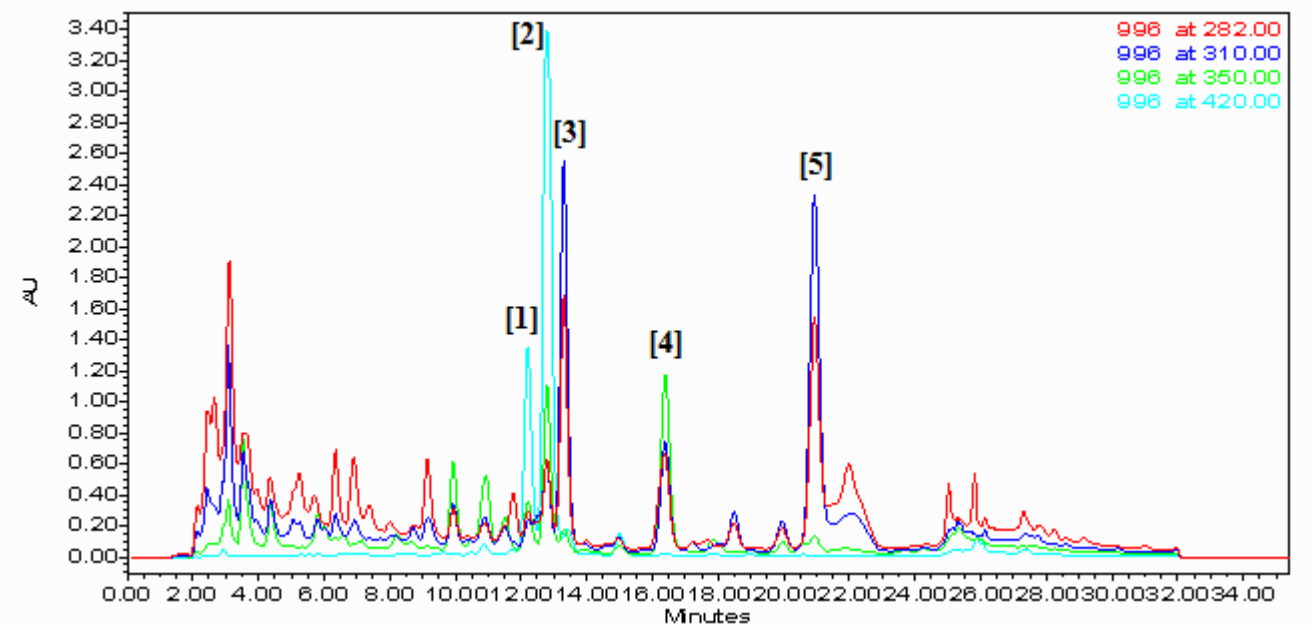


Figure 1
HPLC chromatographic profile of Zingiber phillipseae dichloromethane extract

Identification of Flavone, (1)

m.p. 179 - 180°C, lit.[4] 178 - 179°C; IR (KBr) ν_{max} cm^{-1}: 3437 (OH), 1649, 1622 (C=C), 1595 (C=O); ^{1}H NMR ($CDCl_3$) δ 11.68 (s, 1H, 5-OH), δ 8.12 (d, 2H, *J* = 8.94 Hz, H-2', H-6'), δ 6.98 (d, 2H, *J* = 8.94 Hz, H-3', H-5'), δ 6.52 (s, 1H, 3-OH), δ 6.44 (d, 1H, *J* = 2.06 Hz, H-8), δ 6.32 (s, 1H, *J* = 2.06 Hz, H-6) and δ 3.83 (s,

6H, 4'-OCH_3, 7-OCH_3); ^{13}C NMR ($CDCl_3$) δ 176.16 (C-4), 165.681 (C-7), 161.114 (C-5), 160.800 (C-4'), 156.815 (C-9), 145.699 (C-2), 135.636 (C-3), 129.384 (C-2',6'), 123.160 (C-1'), 114.074 (C-3',5'), 97.883(C-6), 92.186 (C-8), 55.839 (7-OCH_3) and 55.418 (4'-OCH_3); MS *m/z* 314 [M^+].

2. *Biological Study of Dichloromethane Extract*

Brine Shrimp Toxicity Test

A series of *Zingiber phillipseae* and *Zingiber officinale* dichloromethane extracts of 100, 500, 1000, 5000 and 10000 ppm concentration was prepared. Each concentration was triplicated. Dilution of samples concentration were dependent on the toxicity of *Artemia salina* mortality. Potassium dichromate and 10% Tween 80 were used as positive and negative controls respectively. The test was carried out as described by Sam[5]. Cysts were hatched in brine after 16 hours. Nauplii (10) were transferred into sample vials in triplicates for each concentration. After 6 hours and 24 hours, the number of dead nauplii was counted in each vial. LC_{50} value for acute and toxic was calculated by using Reed-Muench Method.

Antioxidant activity assay

ThiocyanateMethod. Assay preparation was according to Jitoe[6] with slightly modified. Dichloromethane extract (4 mg) was introduced to ethanol (4.0 mL; 99.5%), linoleic acid (4.0 ml; 5%) in ethanol (99.5%), phosphate buffer (pH 7.0; 8.0 ml; 0.05 M) and distilled water (3.9 ml) contained in a screw-cap vial (diameter 20 mm x height 75 mm). This was placed in an incubator at 40 – 45^oC in dark. After 24 hours, to the mixture (0.1 mL) was added ethanol (9.7 ml; 75%) and ammonium thiocyanate (0.1 ml; 30%). Then ferrous chloride (0.1 ml; 2 x 10-2 M) in hydrochloric acid (3.5%) was added to the reaction mixture and absorbance was measured after 3 minutes of addition at 500 nm wavelength. The assay was repeated every 24 hours until the control (BHT) reaches to its maximum value.

Antimicrobial activity assay

DiscDiffusion Method. The panel of microorganisms was cultured in appropriate broths at 30^oC overnight. Concentrations of the cultures were adjusted to 10^5 – 10^6 colony forming units per milliliter monitored using a spectrophotometer at a wavelength of 600 nm. The assay was conducted according to a modified method[7,8,9,10]. A series of dichloromethane extract of various concentrations were prepared for preliminary screening: 250, 500, 1000 and 2500 µg/ml. Each concentration was prepared in triplicate. The samples were loaded onto individual Whatman No. 1 filter paper discs (diameter ≈ 6 mm) and placed on inoculated agar. The agar plates were placed in an incubator for 24 hours at 37^oC. Positive activities were showed by clear inhibition zones around the discs. Dilutions were made for further assay to determine the minimum inhibitory concentration (MIC) of the test microorganisms.

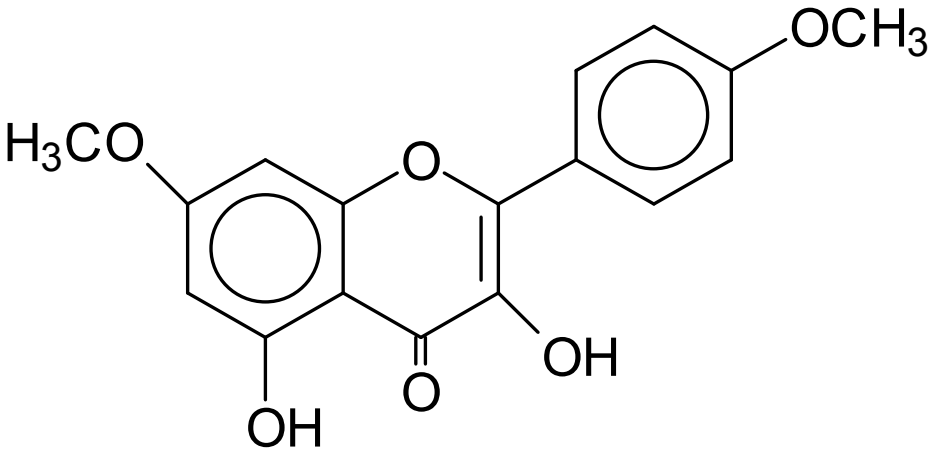
RESULTS AND DISCUSSION

1. *Isolation and Identification of flavones compound*

Extraction of the fresh rhizomes of *Zingiber phillipseae* using dichloromethane yielded 0.11% of crude extract as yellow brownish oil. The crude extract was fractionated by HPLC and five peaks were observed (Fig.1). Compound corresponding to peak 4 subjected to successive chromatography which yielded yellowish needles (**1**). The others fractions were oily yellow liquids but were not purified further.

Compound (**1**), was obtained as yellowish needles, m.p. 179 - 180°C. It showed a [M^+] peak at *m/z* 314 in MS spectrum suggesting the molecular formula $C_{17}H_{14}O_6$. The IR spectrum gave bands at 3437 and 1649 cm^{-1} corresponding to the

presence of hydroxyl and conjugated carbonyl groups respectively. The presence of carbonyl group was supported by the ^{13}C NMR which showed one carbon peak downfield at δ 176.16. The ^{1}H NMR spectrum showed a singlet at δ 3.83 (s, 6H) for two methoxyl groups. Signal at δ 8.12 (d, 2H), δ 6.98 (d, 2H), δ 6.44 (d, 1H), and δ 6.32 (s, 1H) are the aromatic protons. Singlets at δ 11.68 and δ 6.52 were attributed to two hydroxyl groups respectively. The presence of hydroxyl group at C-5 was supported by the bathochromic shift (from 368nm to 421nm) when $AlCl_3$ was added; whereas celullose TLC test of hydroxyl group at C-3 gave yellow in colour under UV and UV/NH_3.



(**1**)

2. ***Bioassays***

Data analysis of mortality of *Artemia salina* using the Reed-Muench Method showed the LC_{50} for acute and toxic levels of *Zingiber phillipseae* dichloromethane extract to be 9,732 ppm and 562 ppm, respectively. The LC_{50} for acute and toxic levels of *Zingiber officinale* dichloromethane extract was 8,927 ppm and 365 ppm, respectively. These indicate that the dichloromethane extract was non-toxic. However, further cytotoxic studies are needed for confirmation.

Its antioxidant activity is shown in Figure 2. The absorbance value of negative control approached 1.2 on the 10th day. The antioxidant activity of *Zingiber phillipseae* dichloromethane extract was higher than the positive control but a bit lower or closer to *Zingiber officinale* dichloromethane extract. The absorbance of *Zingiber officinale* extract was started at 0.011 and increased to 0.111 AU on the 10th day; whereas *Zingiber phillipseae* extract started to absorb from 0.012 to 0.169 AU.

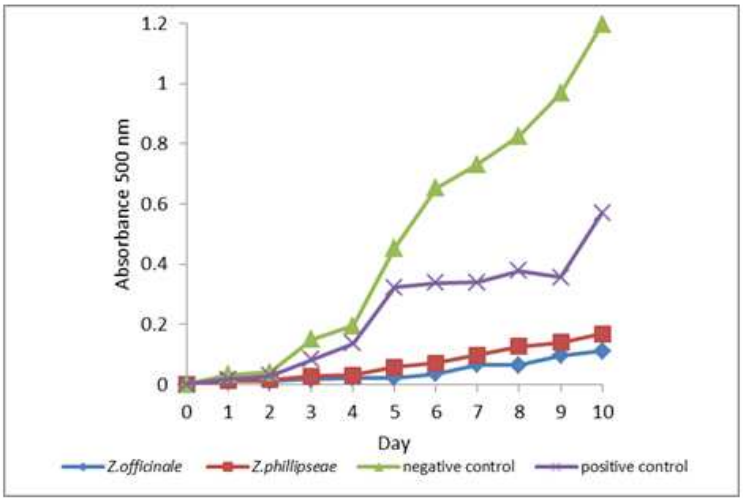


Figure 2
Antioxidant activity of dichloromethane extract: (▲), negative control; (X), positive control;(■), Zingiber phillipseae; (♦), Zingiber officinale.

Zingiber phillipseae dichloromethane extract also showed significant activity against *Staphylococcus aureus* and *Escherichia coli* (Table 2). Both types of bacteria inhibited growth at > 200 µg/ml minimum inhibitory concentration (MIC), compared to *Zingiber officinale* Roscoe dichloromethane extract which required only 450 µg/ml. Both of the species in dichloromethane extracts failed to inhibit the growth of *Candida albicans*.

Table 2
Minimum inhibitory concentration, MIC (µg/ml)

Sample	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Zingiber phillipseae	200	225	> 2500
Zingiber officinale	450	500	> 2500
Streptomycin (reference)	50	50	50

This research only focused on the dichloromethane extract of *Zingiber phillipseae* due to previous studies have indicated that less polar components present in dichloromethane extracts contributed towards the increased activity over the methanol extracts[11]. Currently, there are many studies that examine the phenolic compounds of vegetables, fruits, whole grains, and other plants and their antioxidant role in noncommunicable diseases, such as cancer. Many of these studies suggest that phenolic compounds could become healthy food additives for the prevention of chronic diseases[12]. Results of this study suggest that *Zingiber phillipseae* contains such effective compounds.

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REFERENCES

1. Ibrahim, H. Malaysian Zingiberaceae: Ecological, Morphological and Economic Aspects. *Bull. Heliconia Soc. Inter*. 6 (*1-2*): 4-8, (1992).
2. Theilade, I. & Mood, J. Six new species of *Zingiber* (Zingiberaceae) from Borneo. *Nordic J. Bot.* 19: 513-524, (1999).
3. Hiserodt, R.D., Franzblau, S.G. & Rosen, R.T. Isolation of 6-, 8- and 10-gingerol from ginger rhizome by HPLC and Preliminary Evalution of Inhibition of *Mycobacterium avium* and *Mycobacterium tuberculosis*. *J. Agric. Food Chem*. 46: 2504-2508, (1998).
4. Lee, Y. J. & Wu, T. J. Total synthesis of kaempferol and methylated kaempferol derivatives. *J. Chinese Chem. Soc.* 48: 201-206, (2001).
5. Sam, T.W. Toxicity Testing using the Brine Shrimp: *Artemia salina.* In Colegate, S.M. & Molyneux, R.J. *Bioactive Natural Products: Detection Isolation & Structural Determinatio,* CRC Press, Inc, London,1993, pp.441-456.
6. Jitoe, A., Masuda, T., Tengah, I.G.P., Suprapta, D.N., Gara, I.W. & Nakatani, N. Antioxidant Activity of Tropical Ginger Extracts and Analysis of the Contained Curcuminoids. *J. Agric. Food Chem.* 40: 1337-1340, (1992).
7. Okema, P.O., Mwatha, W.E., Chhabra, S.C. & Fabry, W. The Kill Kinetics of *Azadirachta indica* A. Juss. (Meliaceae) Extracts on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. *African J. Sci. Tech.* 2: 113-118, (2001).
8. Sankar Kumar Dey, Debdulal Banerjee, Sourav Chattapadhyay & Krishnendu Bikash Karmakar. Antimicrobial Activities of some Medicinal Plants of west Bengal. *International Journal of Pharma and Bio Sciences*. 1(3): 1-10 (2010)
9. Gigani Yaseen & Jadhav Sudhakar. Design, Synthesis And Antimicrobial Activity Of 2-Mercaptobenzimidazole Derivatives. *International Journal of Pharma and Bio Sciences.* 1(4): 281-286, (2010).
10. M. Rama Prabha & K. Vasantha. Phytochemical And Antibacterial Activity Of Calotropis Procera (Ait.) R. Br. Flowers. *International Journal of Pharma and Bio Sciences.* 3(1): 1-6 (2012).
11. Habsah, M., Amran, M., Mackeen, M.M., Lajis N.H., Kikuzaki, H., Nakatani, N., Rahman, A.A., Ghafar & Ali, A.M. Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. J. Ethnopharmacol 72: 403-410, (2000).
12. Finley, J. W., Kong, A. N., Hintze, K. J., Jeffery, E. H., Ji, L. L., Lei, X. G. Antioxidants in foods: state of the science important to the food industry *J. Agric. Food Chem*. 59 (*13*): 6837– 6846, (2011).