PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF
KAEMPFERIA GALANGA

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PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF KAEMPFERIA GALANGA

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This project is submitted in partial fulfilment of the requirements for the degree of Bachelor of Science with Honours (Resource Chemistry)

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2004

- Plant Extracts
- Leaves - Anatomy
- Plant Leaves
DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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ABSTRACT

A phytochemical study on the rhizomes of *Kaempferia galanga* (Zingiberaceae) was carried out. In this study extraction, fractionation and purification of this sample were carried out. There are three fractionation obtain from this sample known as 3CL1, 3CL2 and 3CL3. Fractionation and purification on this sample were carried out by thin layer chromatography, column chromatography and preparative thin layer chromatography. Toxicity test on *Artemia salina* showed that only fractions 3CL2 gave LD₅₀ values of 100 μg/ml while chloroform extract, fraction 3CL1 and fractions 3CL3 gave values of LD₅₀ more than 100 μg/ml.

Key words: *Kaempferia galanga*, fractionation, purification, toxicity test
ABSTRACT

Kajian jilokinia telah dijalankan ke atas rhizome Kaempferia galanga (Zingiberaceae). Dalam kajian ini, pengekstrakan, pemfraksian dan penulenian telah dilakukan. Tiga fraksi telah didapatkan daripada sampel ini dikenali sebagai 3CL1, 3CL2 dan 3CL3. Pemfraksian dan penulenian telah dilakukan dengan menggunakan kaedah kromatografi lapisan nipis, kromatografi turus dan kromatografi lapisan nipis persediaan. Ujian ketoksikan ke atas larva Artemia salina menunjukkan hanya fraksi 3CL2 memberikan nilai LD_{50} pada kepekatan 100 μg/ml manakala bagi ekstrak kloroform dan fraksi 3CL1 dan 3CL3 nilai LD_{50} melebihi 100 μg/mL.

Kata kunci: Kaempferia galanga, pemfraksian, penulenian, ujian ketoksikan
CHAPTER ONE  INTRODUCTION

1.1 Literature review

Herbs have been found in a wide range of usage such as health and pharmaceutical properties. Our country is considered to have about 20000 plant species, 2000 plant species and reported to have medicinal values (Indu Bala Jaganath et al, 2000).

Halijah Ibrahim et al, 2000, reported at least 16 species from zingiberaceae family are frequently utilized by traditional healers for various purposes such as traditional medicine, food and miscellaneous uses that can be found from both cultivated and wild plants (Halijah Ibrahim et al, 2000).

Rhizome from Zingiberaceae famili has been found to contain active compound that effective for the treatment of thrombosis, sea sickness, migraine and rheumatism (Puangpen Sirirugsa, 1999).

According to S Vimala et al, 1999, several Zingiberaceae species used in Malaysian traditional medicine contain naturally occurring non-toxic compounds that can contribute in the development of cancer prevention methods at the tumour-promoting stage.

Two plant families of Zingiberaceae and Rutaceae, were identified to be promising sources for highly effective anti-tumour promoters when 40 methanol extract from Thailand that used in various purpose were screened for the inhibitory activity toward Epstein-Barr virus activation (A. Murakami et al, 1994).
*Kaempferia galanga* from the family of Zingiberaceae have been reported to have medicinal values. *Kaempferia galanga* is a very short herb contains white-purple flowers in the center of the plant and the rhizome of this plant is strongly branched, aromatic and colourful. The distribution of this plant are mainly in India, China, Southeast Asia, Malaysia, Indonesia and Singapore. This plant could be applied as flavouring in food, medicine and cosmetic product (Indu Bala Jaganath *et al.*, 2000).

According to James A. Duke, 1985, the essential oil of this plant contains *n*-pentadecane, ethyl-*p*-methoxycinnamate, ethyl cinnamate, careen, camphene, borneol, and *p*-methoxystyrene (James A. Duke, 1985).

This plant also reported to contain 4-butylmenthol, *β*-phellandrene, *e*-terpineol, dihydro-*β*-sesquiphellandrene, pentadecane and 1,8-cineol. The rhizome is also reported to display cytotoxic properties (Garnot Katzer, 2001).

Ethylcinnamate that was isolated and purified from the rhizome of the plant was identified as the major compound contributing to the vasorelaxant activity due to the inhibition of calcium influx through the voltage and receptor-operated channels (Rozana Othman *et al.*, 2002).

When the oral administration of the extracts (20 mg/day) *K. galanga* had been applied to high colestrol white wistar rats over a period of 4 weeks it showed that it can effectively lowered the serum and tissue levels of total cholesterol, triglycerides,
phospholipids and significantly increased the serum levels of high density lipoproteins (HDL) cholesterol indicated that these plants in various lipid disorders especially atherosclerosis (Achuthan CR, et al, 1997).

Ethyl esters cinnamate and p-methoxycinnamate that was extract from this plant found to caused pronounced mortality to the larvae in insecticidal activity studies (Chillwan Pandji et al, 1993).

The ethanolic extract from *Kaempferia galanga* showed central nervous system depression such as a decrease in motor activity and respiratory rate, and a loss of screen grip and analgesia when test against rats using hippocratic screening test (D. Kanjanapothi, et al, 2003).

The methanolic extract of *K. galanga* showed anti-ulcer activity when tested in rats using various experimental models, which include ethyl acetate or hydroloric acid, restraint water immersion stress, pylorus ligation, and indomethacin-induced gastric lesions (Kesaraporn Wanajak, 1999).

1.2 Objectives

The main purpose of this research is to studies phytochemical and biological activities of *Kaempferia galanga* and the objectives of this research are to isolate new compound and bioactive compound from the plant and also to study the biological activity against brine shrimp toxicity.
CHAPTER TWO  MATERIALS AND METHODS

2.1 General procedures

Gas Chromatography and Mass Spectrometry on a Shimadzu GC-MS (MSQF-1000) were used to identify molecular structure of the compounds isolated from the plant. Thin layer Chromatography and Preparation Thin Layer Chromatography plate (Merck, Kieselgel 60F254, 0.25 mm) was used in the separation technique. Silica gel 60 (Merck, 230-400 mesh) was used in column chromatography. Cytotoxic assay using brine shrimps were prepared for biological activity.

2.2 Plant materials

*Kaempferia galanga* (3kg) was obtained from the market and identified and the rhizomes were air dried at room temperature and grounded to a fine powder prior to extraction.

2.3 Toxicity Test

2mg of chloroform extract, fraction 3CL1, 3CL2 and 3CL3 were diluted with 2ml chloroform. About 5 μl, 50μl and 500μl of the solution from each of the sample was put in the test tube. Each of the samples was done in triplicate. Each of the solution was evaporated to dryness using rotovapour and added with 5ml seawater. 2ml of each solution was transferred into *NUNC multidish*. 10 larvae of *Artemia salina* were put into each of the *NUNC multidish*. First observation was done in first three hour and final result was getting after 24 hour. A number of larvae that still survive are counted and LD₅₀ was determined. Control test was done using same method and using seawater.
2.4 Extractions and Isolation

Dried rhizomes powder (350 g) was extracted with methanol (3 times). The extracts were combined and evaporated to dryness under pressure 50°C to give 6 g of crude extract. The methanol extract was dissolved in methanol: water to ca. 100 ml, basified with 27% NH₃ solution to pH 11-12, and partition with CHCL₃ (150 ml x 3 times). The CHCL₃ extract was concentrated to give a brown residue (300mg). The residue (100mg) was applied to a thin layer chromatography (TLC) and eluted with solvent system CHCl₃: MeOH (9:1) that give three components when identified with UV light at 254 nm (UVP model cc-10) and the position of components were marked (appendix 1). The plate was sprayed with detector (5% of H₂SO₄ in MeOH) and the positions of visible components were recorded. Rₜ value for each component are identified and showed in table 1.

Table 1: Rₜ value for each component that contain in the rhizomes of *Kaempferia Galanga*

<table>
<thead>
<tr>
<th>Spots</th>
<th>Rₜ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>0.76</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Chloroform extract (200mg) then purified with column chromatography with system solvent CHCL₃:MeOH (9:1) and CHCl₃ to give three fraction: fraction 3CL1 (53.4mg) (appendix 2), fraction 3CL2 (62.3 mg), fraction 3CL 3 (50mg) (appendix 3). Fraction 3CL2 was further purified on prep. TLC (silica gel 60 F₂₅₄, Merek) using CHCl₃: MeOH (9:1) to give compound 1 (appendix 4). The molecular weight of the compound then identified using Gas Chromatography (Shimadzu GC-MS (MSQP-1000)).
CHAPTER 3 RESULT AND DISCUSSION

3.1 Extraction and Isolation

The chloroform extract gave 3 fraction using TLC with solvent system CHCl₃-MeOH (9:1) (appendix 1). Chloroform extract (200mg) that isolated with Column Chromatography using CHCl₃-MeOH (9:1) and CHCl₃ gave three fraction; 3CL1 (53.4mg) (appendix 2), 3CL2 (62.3 mg), 3CL3 (50mg) (appendix 3). 3CL2 was the main compound because it biologically active (LD₅₀ values of 100µg/ml) compared to other fraction. 3CL2 was further purified and separated using preparative TLC (silica gel 60 F₂₅₄, Merck) using CHCl₃: MeOH (9:1) to gave compound 1 with Rₜ value =0.6 (appendix 4). Identification of molecular weight of compound 1 using Gas Chromatography gave values 207.

Structure identification using spectroscopy method such as infra red (IR), ultra violet (UV), nucleus magnetic resonans (NMR) (¹H & ¹³C) and melting point was not done because insufficient amount of the compound.

3.2 Toxicity test

Toxicity test on Artemia salina showed that all compound displayed cytotoxic activity (Gernot Katzer, 2001). Fractions 3CL 2 was a most active compound give LD₅₀ values of 100 µg/ml while fraction 3CL1 and fractions 3CL3 and also chloroform extract gave values of LD₅₀ more than 100 µg/ml. Table 2 and diagram 1 showed average number of Artemia salina larvae died in 24 hours.
**Table 2:** Average percentage of *Artemia salina* that died after 24 hour in concentration of 1, 10 and 100 µg/ml

<table>
<thead>
<tr>
<th></th>
<th>1µg/ml</th>
<th>10µg/ml</th>
<th>100µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform extract</td>
<td>0</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>3CL 1</td>
<td>10</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>3 CL 2</td>
<td>0</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>3CL 3</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
Figure 1: Average percentage of Artemia salina larvae died in different concentrations of rhizomes Kaempferia galanga extract.
Figure 2: Summary for extraction and isolation and bioassay for fraction obtained from mushrooms *Fomitopsis gaumeri*.
CHAPTER FOUR  CONCLUSION AND SUGGESTION

Fraction 3CL2 from chloroform extract give compound 1 with $R_f = 0.6$. Identification of molecular weight of the compound using Gas Chromatography gave value 207. Structure identification of the compound 1 was not done because insufficient amount of the compound. Toxicity test that was done to Artemia salina showed only fraction 3CL2 gave LD$_{50}$ concentration in 100µg/ml. Fraction 3CL1 and 3CL3 gave LD$_{50}$ more than concentration 100µg/ml.

Further research of Kaempferia gelanga should be done because this species was identified contained biological active compound that have potential to make as a medicine.
REFERENCES


Miss Kesaraporn Wanajak, 1999. Anti-Gastric Ulcer Activity of *Kaempferia galanga* Linn.


Appendix 1: Chloroform crude extract from rhizomes of Kaempferia galanga in system solvent CHCl₃:MeOH (9:1)

Appendix 2: Fraction 3CL1 and 3CL2 in system solvent CHCl₃:MeOH (9:1)
Appendix 3: Fraction 3CL3 in system solvent CHCL3:MeOH (1:1)

Appendix 4: Compound 1 from fraction 3CL2 in system solvent CHCL3:MeOH (9:1)