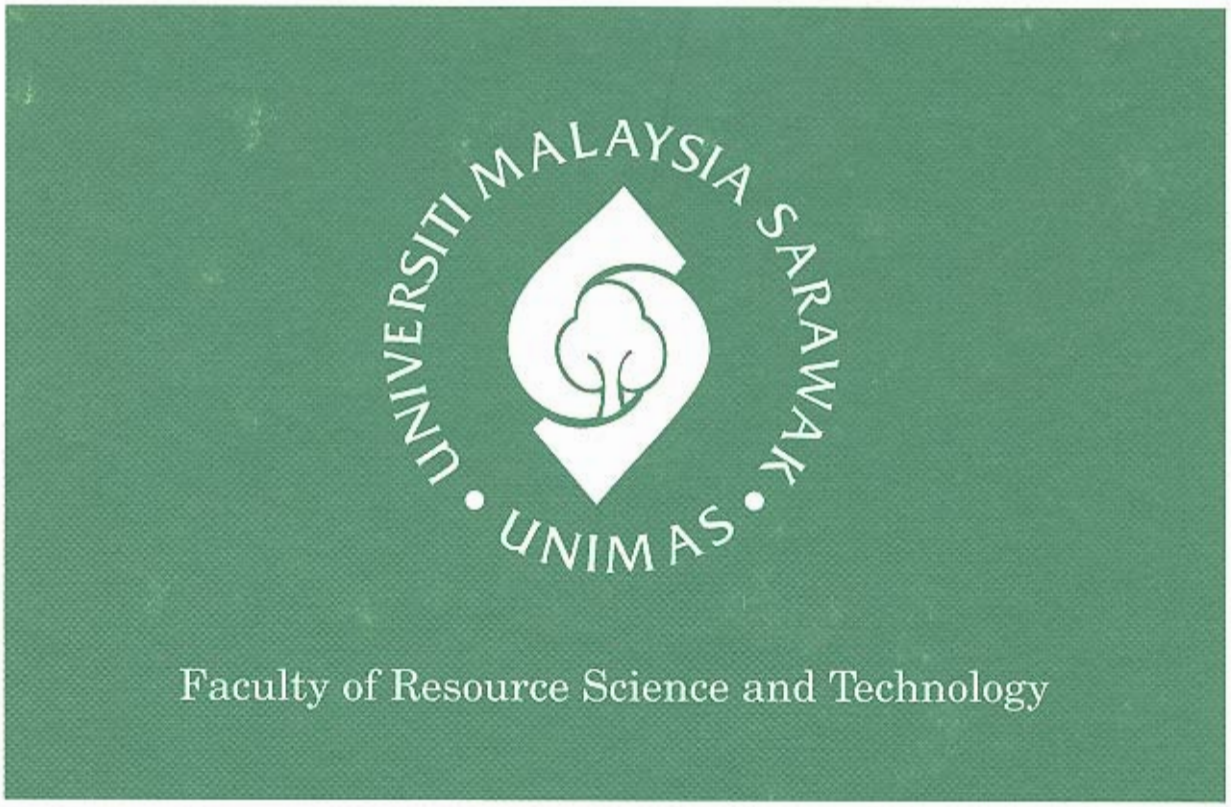

PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF *KAEMPFERIA GALANGA*

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Bachelor of Science with Honours
(Resource Chemistry)
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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
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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 chromathography, column chromathography and preparative thin layer chromathography. Toxicity test on *Artemia salina* showed that only fractions 3CL2 gave LD_{50} values of 100 μg/ml while chloroform extract, fraction 3CL1 and fractions 3CL 3 gave values of LD_{50} more than 100 μg/ml.

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

ABSTRACK

Kajian fitokimia telah dijalankan ke atas rhizome Kaempferia galanga (Zingiberaceae). Dalam kajian ini, pengekstrakan, pemfraksian dan penulenan telah dilakukan . Tiga fraksi telah didapati daripada sampel ini dikenali sebagai 3CL1, 3CL2 dan 3CL3. Pemfraksian dan penulenan telah dilakukan dengan menggunakan kaedah kromatografi lapisan nipis, kromatografi turus dan kromatogfafi 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,penulenan,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 zingeberaceous 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 Zingeberaceae famili has been found to contain active compound that effective for the treatment of thrombosis, sea sickness, migraine and rheumatism (Puangpen Sirirugsa, 1999).

Accroding 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, South-east Asia, Malaysia, Indonesia and Singapore. This plant could be applied as flavouring in food, medicine and cosmetic product (Indu Bala Jaganath *et al*, 2000).

Accroding 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, α-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 tothe larveae 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 Chromathography and Mass Spectrometry on a Shimadzu GC-MS (MSQP-1000) were used to identify molecular structure of the compounds isolated from the plant. Thin layer Chromathography and Preparation Thin Layer Chromathography plate (Merck, Kieselgel $60F_{254}$, 0.25 mm) was used in the separation technique. Silica gel 60 (Merck, 230-400 mesh) was used in column chromathography. 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 LD_{50} 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% NH_3 solution to pH 11-12, and partition with $CHCL_3$ (150ml x 3 times). The $CHCL_3$ extract was concentrated to give a brown residue (300mg). The residue (100mg) was applied to a thin layer chromathography (TLC) and eluted with solvent system $CHCl_3$: 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 H_2SO_4 in MeOH) and the positions of visible components were recorded. R_f value for each component are identified and showed in table 1.

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

Spots	R_f values
1	0.5
2	0.76
3	0.9

Chloroform extract (200mg) then purified with column chromathography with system solvent $CHCL_3$:MeOH (9:1) and $CHCl_3$ 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 F_{254}, Merck) using $CHCl_3$: MeOH (9:1) to give compound 1 (appendix 4). The molecular weight of the compound then identified using Gas Chromathography (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 $CHCL_3$-MeOH (9:1) (appendix 1). Chloroform extract (200mg) that isolated with Column Chromathography using $CHCl_3$-MeOH (9:1) and $CHCl_3$ 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 (LD_{50} values of 100μg/ml) compared to other fraction. 3CL2 was further purified and separated using preparative TLC (silica gel 60 F_{254}, Merck) using $CHCl_3$: MeOH (9:1) to gave compound 1 with R_f value =0.6 (appendix 4). Identification of molecular weight of compound 1 using Gas Chromathography gave values 207.

Structure identification using spectroscopy method such as infra red (IR), ultra violet (UV), nuckleus magnetic resonans (NMR) (^1H & ^{13}C) 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 LD_{50} values of 100 μg/ml while fraction 3CL1 and fractions 3CL3 and also chloroform extract gave values of LD_{50} 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

	Average number of *Artemia salina* died (%)		
	1µg/ml	10µg/ml	100µg/ml
Chloroform extract	0	10	20
3CL 1	10	20	40
3 CL 2	0	10	50
3CL 3	10	20	20

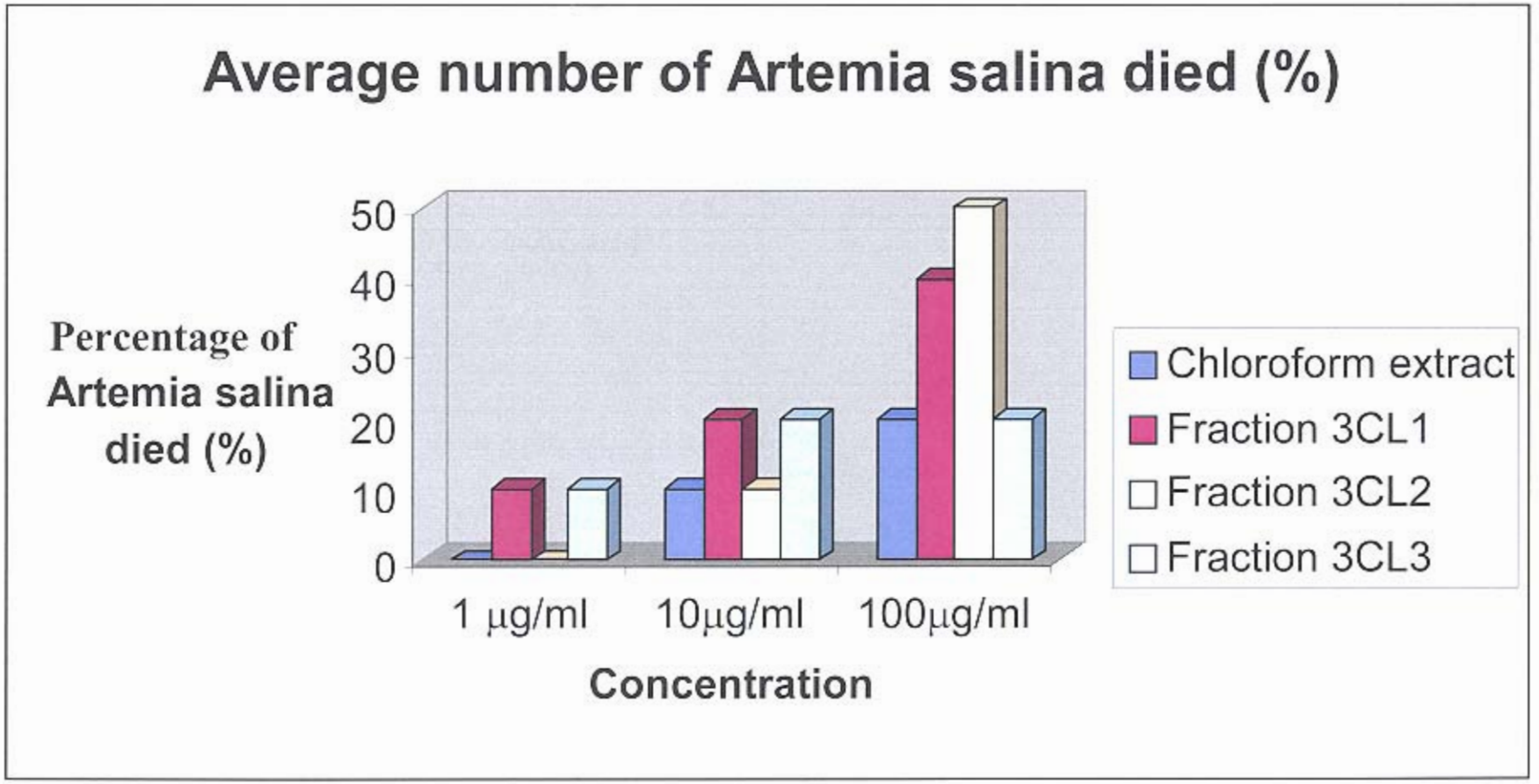

Figure 1: Average percentage of *Artemia salina* larve died in different concentration of rhizomes Kaempferia galanga extract

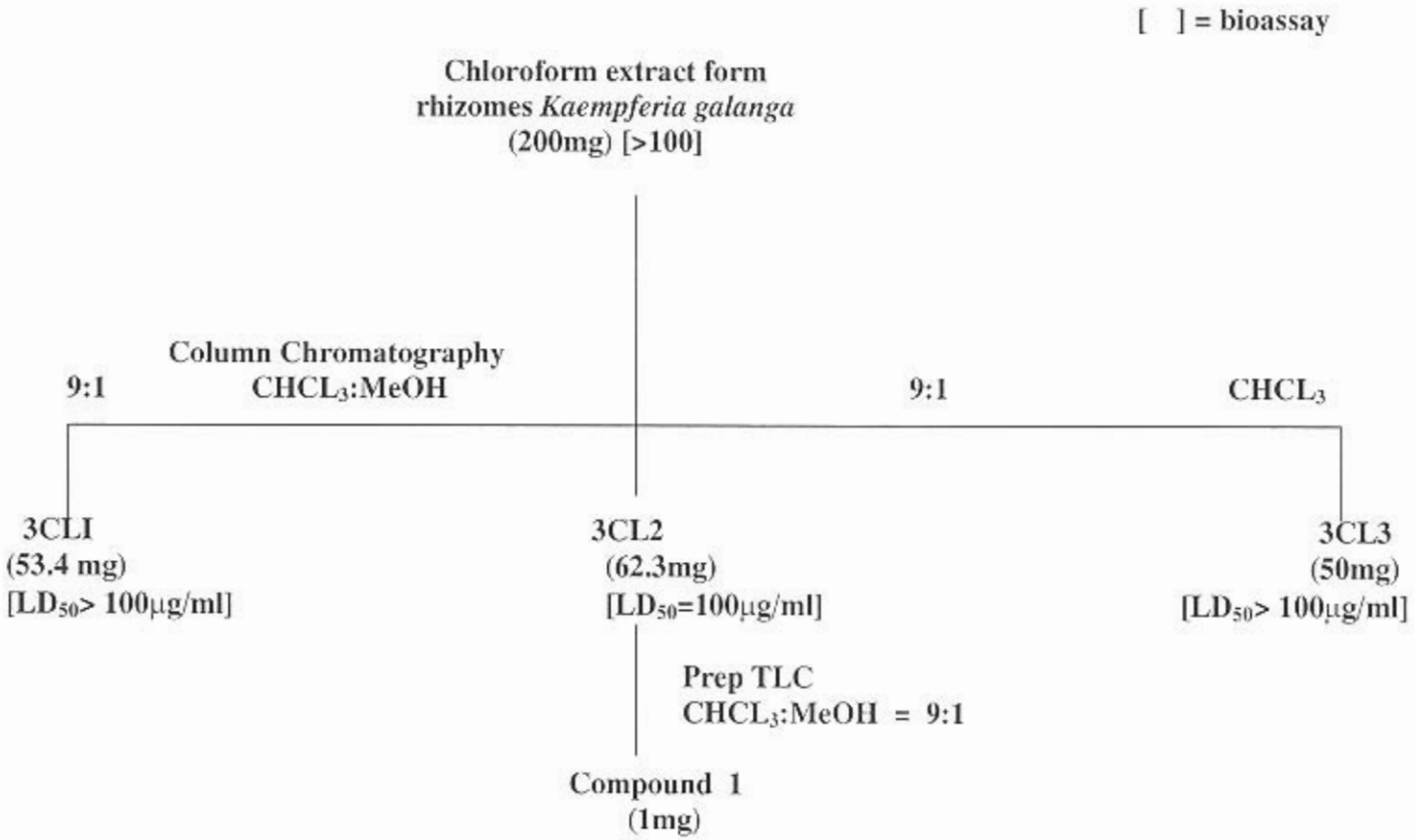

Figure 2 : Summary for extraction and isolation and bioassay for fraction obtain from rhizomes *Kaempferia galanga*

CHAPTER FOUR CONCLUSION AND SUGGESTION

Fraction 3CL 2 from chloroform extract give compound 1 with R_f =0.6. Identification of molecular weight of the compound using Gas Chromathography gave value 207. Structure identification of the compound 1 was not done because insufficient amount of the compund. 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 galanga* should be done because this species was identified contained biological active compound that have potential to make as a medicine.

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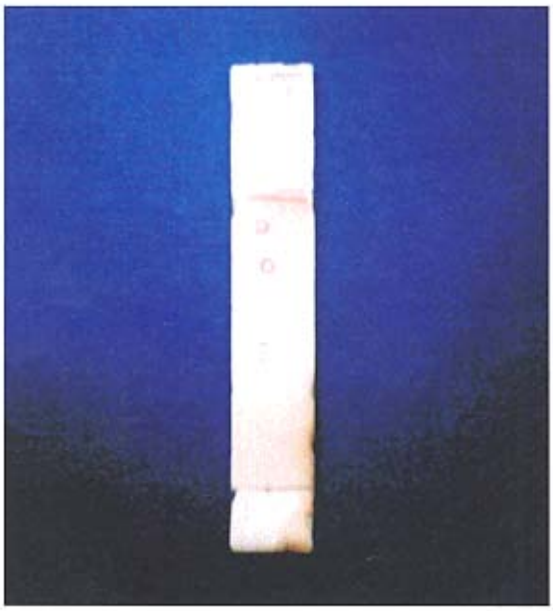
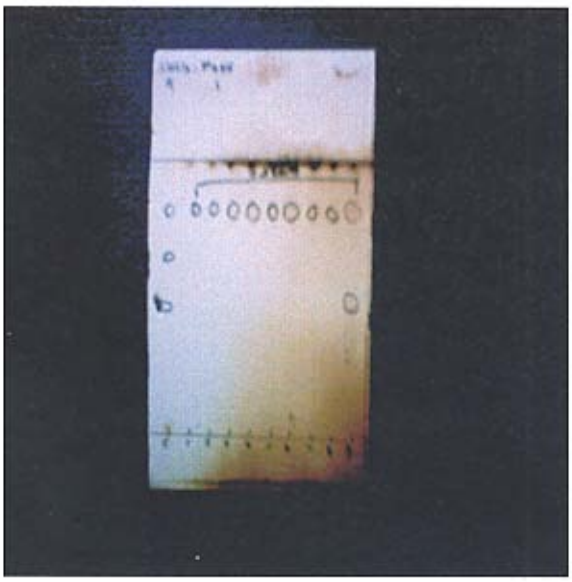
APPENDIXES

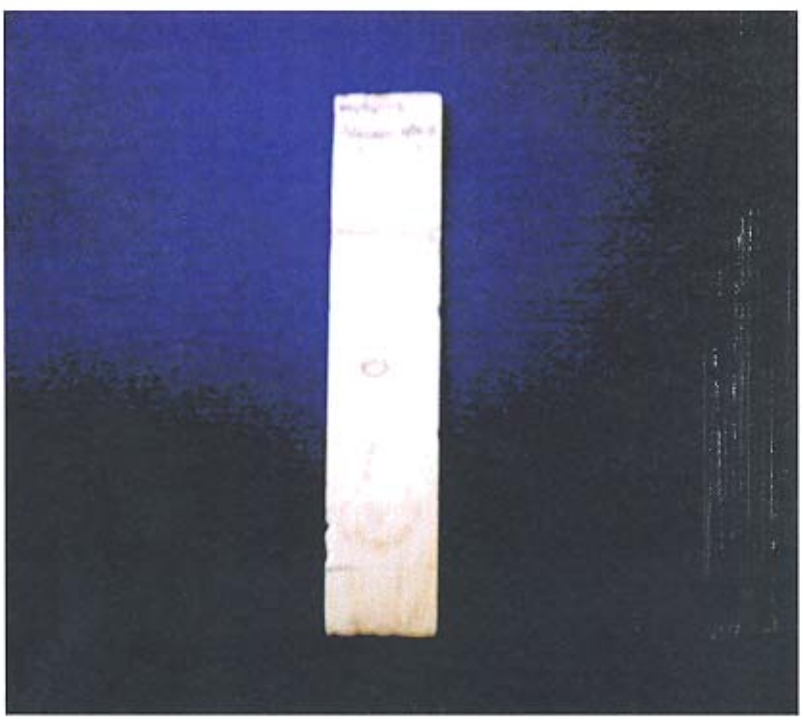


Appendix 1: Chloroform crude extract from rhizomes of Kaempferia galanga in system solvent $CHCL_3$: MeOH (9:1)



Appendix 2 : Fraction 3CL1 and 3CL2 in system solvent $CHCL_3$:MeOH (9:1)

Appendix 3 : Fractiom 3CL3 in system solvent $CHCL_3$:MeOH (1:1)



Appendix 4 : Compound 1 from fraction 3CL2 in system solvent $CHCL_3$MeOH (9:1)