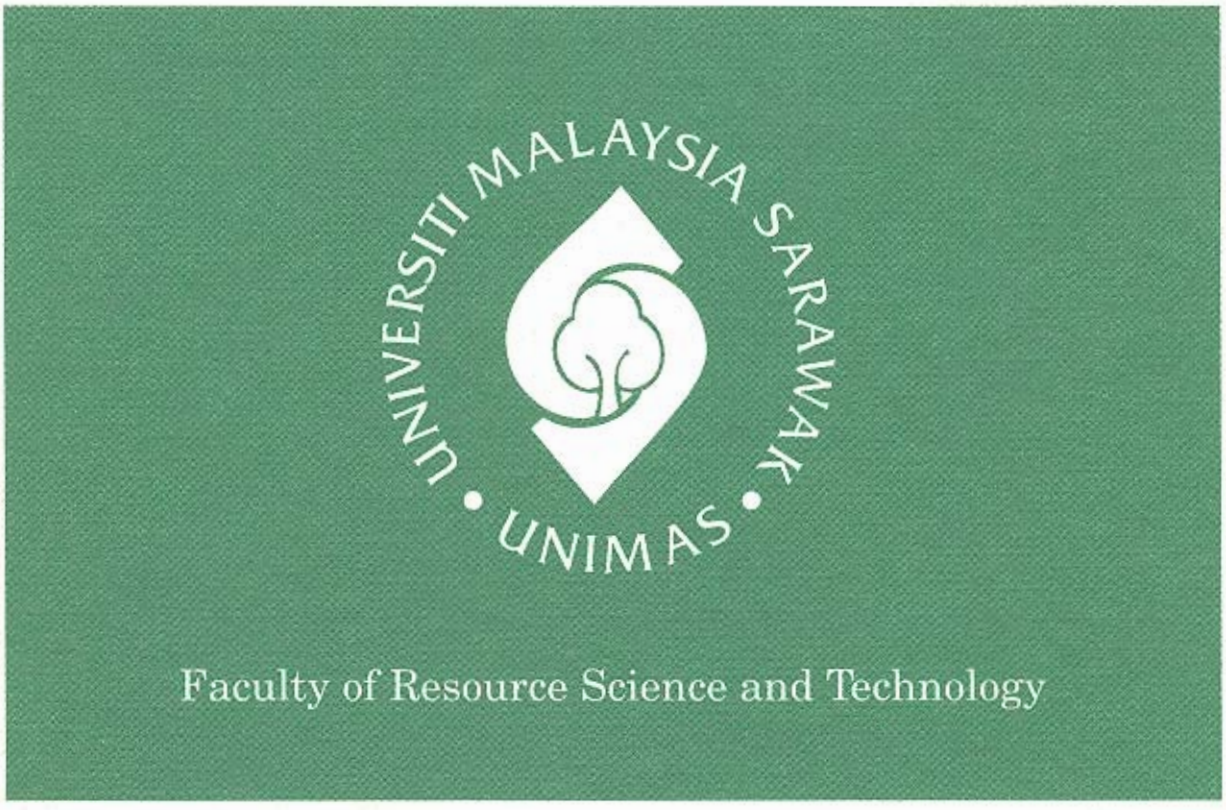

STUDY ON ETHANOLIC EXTRACT OF *ERECHTITES VALERIANIFOLIA* (COMPOSITAE)

Mohd. Nurhafezam Mazuki

Bachelor of Science with Honours
(Resource Chemistry)
2005

STUDY ON ETHANOLIC EXTRACT OF *ERECHTITES VALERIANIFOLIA* (COMPOSITAE)

MOHD NURHAFEZAM MAZUKI

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

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2005

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.

Mohd Nurhafezam Mazuki
Program of Resource Chemistry
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ACKNOWLEDGEMENT

First and foremost, I pray to Allah swt. for all the gift and blessing He gave to me from the beginning. Without His Mercy, I would never finish this project. I would like to thank my supervisor, Dr Kamarul'Ain, for accepting me and guiding me through out the project and keep correcting my mistakes. I also would like to thank my friends (Hamdan, Muhammad, Hanafi, Nasri etc) who always there for me eventhough I was not always there for them. I would also thank the lab assistants, who prepare things for me and always makes me feel comfortable in the lab, especially Mr Rajuna, Mr Jahina, and others. Thank you sincerely, hopes we could meet and work together again in future. My deepest gratitude to my parent and my family, who always lift me up when I was feeling down and never discourage me from doing what I believe in doing and for that, I really thank you. May Allah bless you all and guide you in every thing that you do. Ameen.

TABLE OF CONTENTS

Abstract

A study on ethanolic extract of *Erechtites valerianifolia* was carried out. In this study, extraction, phytochemical screening and fractionation, using column chromatography were carried out. Phytochemical screening showed the presence of flavonoid in samples of *Erechtites valerianifolia*. The isolation and purification on this sample were carried out using thin layer chromatography and column chromatography. Toxicity test of crude extract of *Erechtites valerianifolia* on *Artemia salina* gave LC_{50} value of 100μg/mL.

Keywords: Erechtites valerianifolia, Compositae, fractionation, phytochemical screening, toxicity test

Abstrak

Kajian menyeluruh mengenai ekstrak ethanol dari tumbuhan Erechtites valerianifolia (Compositae) telah dijalankan. Dalam kajian ini, pengekstrakan, penyaringan fitokimia, dan pemfraksian, menggunakan kromatografi turus telah dilakukan. Keputusan penyaringan fitokimia menunjukkan kehadiran flavonoid telah dikesan dalam sampel Erechtites valerianifolia. Proses pemisahan dan pemulenan telah dijalankan dengan menggunakan kromatografi lapisan nipis dan kromatogarfi turus. Ujian ketoksikan ke atas larva Artemia salina menunjukkan ekstrak kasar Erechtites valerianifolia memberikan nilai LC_{50} pada kepekatan 100μg/mL.

Kata kunci: Erechtites valerianifolia, Compositae, pemfraksian, penyaringan fitokimia, ujian ketoksikan.

CHAPTER 1

1.0 Introduction

Native people around the world have been known to use the best of nature in every aspect of their life. They use plant to cure and help them in their daily life, since ancient time. Before the availability of modern medicine, the traditional medicine involving plants and animals are widely used to cure many diseases and sickness.[1] Even now, when there is the availability of modern medicine, many of the native people and even modern people still believe in the healing properties of medicinal plant. Because of this reason, many scientists have put on continual efforts in research to explain the uses of medicinal plants in traditional medicine in a scientific way. Nowadays, there are at least 125 new drugs that have been successfully synthesized to replace the natural resources.[1] Many of the medicinal plant properties are still mysterious to science world, so research about their chemical component and bioactive compounds increased by day. There are many ways to study the chemical properties of these plants. One of those is, to study in depth the properties of the extract as was chosen by many researchers nowadays. This present project will adopt similar principles of studying the extract obtained using alcoholic solvent followed by several chromatographic and simple tests procedures. The plant selected for this study is *Erechtites valerianifolia* (Wolf) DC from the family Compositae. To the best of our knowledge, no report has been published regarding this plant. For this reason, the study of this plant is crucial. Basic extraction technique and the chemical component of the crude extract will be monitored using analytical TLC and Column Chromatography. Functional group will be determined using FTIR method. Bioassay test will be conducted to determine the cytotoxicity of

the crude extract using *Artemia salina* or Brine Shrimp. After several test, hopefully we will get a major compound and be able to isolate it and determine the structure of the component.

1.1 Literature review

Compositae is one of the largest flowering plant in the planet. According to Kirtikar,[2] there are 13000 species in 900 genera in this family while other said that it has 1,100 to 2000 genera and more than 20,000 species in this family.[3-6] These plants predominantly herbaceous, although woody species also exist.[7] Compositae family members are found in diverse habitats; and some were even detected in the most inhospitable habitats such as desert and salt marshes.[8] The family is a rich source of powerful insecticide and industrial chemicals, e.g., pyrethrum (*Chrysanthymum*) and rubber (*Guayule*).[9] The members of this family of plants are also widely used as herbs and medicine for people, used in religious practices by natives and commonly grown for their attractives flowers.[10]

Erechtites is one of the genera in the Compositae family and also known as *Senecio*. *Erechtites* are commonly known as burnweed by the West. Several species of this genus that has been studied previously include *E. glomerata*, *E. minimal*, *E. agrestis*, and *E. cacalioides*.

Erechtites valerianifolia (Wolf) DC is also known as *Senecio valerianifolia* Wolf. Common names for this plant are *asang-asang* (Bidayuh) and tropical burnweed. This plant is usually found in low lands, but also found in higher altitude, such as in Fiji (1,323m above sea level).[11] *E. valerianifolia* is an annual herb of 0.5-2.5m tall; the leaves are usually serrate, with 4-25cm long and 2-10cm wide.[12] The color of the flower of this plant was to be reported in several include, magenta, purple[13], cream and yellow[14]. *E. valerianifolia* propagates through seeds and common in open, disturbed places[15] often in montane taro crops.[16] This plant also used for

treatment of injuries cause by formic acid burn (unpublished data). [17] To best of our knowledge, data on this medicinal plant properties and chemical compounds is very lacking.

Figure 1: *Erechtites valerianifolia* with yellow colored flower

Figure 2: *Erechtites valerianifolia* with purple colored flower[18]

CHAPTER 2

2.0 Material and method

2.1 General

Kitchen Blender model National MX-895M and Mallincroft ethanol analytical grade was used for extraction. Solvent from extract was removed using Rotary Evaporator. Analytical TLC was performed using Merck Plastic coated silica gel 0.25mm. Solvent used for analytical TLC was analytical grade, Mallincroft absolute EtOAc and cyclohexane, with ratio (1:3). For 2-D TLC, Merck Plastic coated silica gel 0.25mm was used. Column Chromatography (CC) was performed using a column of the size 30 mm diameters by 600mm length. Silica gel used for CC is Merck Silica Gel 60 (0.040-0.063mm) 200-400 mesh. For functional group identification, a Shimadzu FTIR-8201PC Fourier Transform Infra-Red Spectrometer was used.

2.2 Plant material

Plant sample (*Erechtites valerianifolia*) was collected in August, 10, 2004 from the area of Kg meranek, Kota Samarahan, Sarawak. The sample was air dried for one month.

2.3 Extraction

Dried sample (100 g) was extracted using ethanol (500 ml. x 2) by grinding with a blender. The extract was let to stand for a few minutes to let the big particle to settle down and then filtered through filter funnel plugged with cotton wool. The residue was discarded and the solvent from the filtrate was removed using a Rotary Evaporator and the mass of crude extract was obtained.

2.4 Analytical Thin Layer Chromatography

2.4.1 Analytical TLC was used to detect components in the extract. Solvent used to develop TLC was a mixture of EtOAc and cyclohexane in the ratio of (1:3) v/v respectively. UV light, iodine vapor and 'vanillins dip' (see below) was used as visualizing technique.

$$R_f = \frac{\text{distance from the point of application of the sample to one of the components}}{\text{distance from the point of application of the sample to the solvent front}}$$

2.4.1 Iodine Vapor test

The iodine crystal were placed into the Pasteur pipette plugged with cotton wool and by blowing it directly onto the TLC, brownish yellow spots appeared.

2.4.2 Vanillin Dipping

Preparation of Vanillin solution: 1.0 g of vanillin was dissolved in 100.0 ml of ethanol in an Erlenmeyer flask. Then 1.0 mL of H_2SO_4 was slowly added to the solution with stirring. The combined solution was put into a container.

2.4.3 Procedure

Developed TLC to be visualized was dipped into the vanillin solution and dried using hairdryer until some colored spots appeared.

2.5 2-Dimensional TLC (2D-TLC)

2D TLC was used to detect any flavonoids compound existed in the crude extract. 2.0 mg of crude extract of *Erechtites valerianifolia* was used for this test. Two types of solvent were used to develop this TLC. The first one is a mixture of n-butanol, distilled water, and glacial acetic acid (BAW) with ratio of (4:1:5) v/v respectively for the first solvent. The second solvent was 15% acetic acid for the second.

2.5.1 Solvent Preparation for 2-D TLC

In 2D TLC, two types of solvent were used for developing. The first solvent consists of 200 ml. of butanol, 50 ml. of acetic acid and 250 ml. of water. These solvents were combined and put into separating funnel, well shake and left to stand for few minutes until two layers were observed. The upper layer was collected for developing the TLC, while the bottom layer was discarded. For the second solvent, 500 ml. of distilled water was mixed with 75 ml. of acetic acid to give 15 % of acetic acid solution. All experiments were done in the fume hood.

2.5.2 2-D TLC Testing Procedure

2.0 mg of crude extract (*Erechtites valerianifolia*) was taken and dissolved with 80% of methanol. Then, by using capillary tube, all diluted crude extracts were spotted onto the pre-measured TLC plate making a single spot.

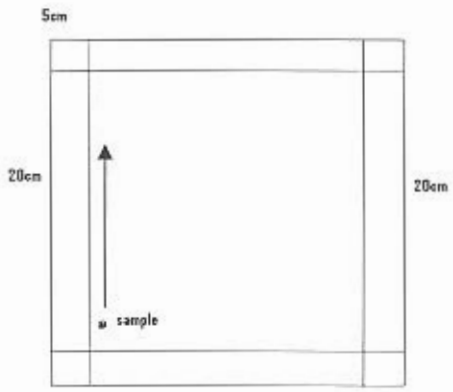

Figure 3

Then 2-D TLC spotted with sample was then developed using the first solvent until the solvent reached the pre measured marked. This process took approximately three hours. TLC paper was let to air dry and then developed again using second solvent as shown in Figure 4. This process took approximately another seven hours.

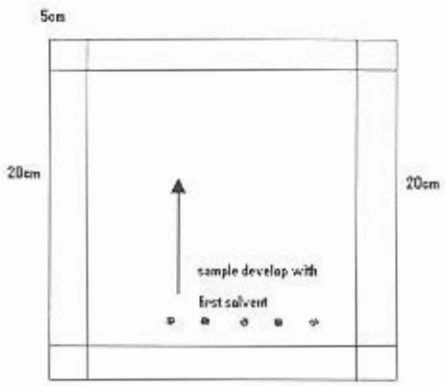

Figure 4

Then the TLC paper was let to dry and visualized under UV lamp with wavelength of 306 nm. Retention factor, R_f for each group of fluorescence spots were then determined.

2.6 Column Chromatography

Column Chromatography (**CC**) was used to separate the compounds detected on analytical TLC. Different ratio of cyclohexane and EtOAc was used for fractionation (**Table 1**). 10 mL of each fraction was taken. Methanol was used at the end to flush out any remaining compound in the column. Analytical TLC was used throughout the process and any similarities with any subsequence fractions were combined and mass for each fraction were obtained.

2.6.1 Column Preparation

Column with the size of 60.0 cm in length and 3.0 cm in diameter was used. It was rinsed by pouring acetone and the selected solvent system before being used. 100 g of silica gel were used to pack the column. Then cyclohexane was added to silica gel to make silica slurry which then was transferred into the sintered column. Some cyclohexane was added to the column and let it drip into a beaker to ensure all the air bubbles have been displaced. The column then was left to stand for approximately 24 hours.

2.6.2 Sample preparation

5.0 g of crude extract of *Erechtites valerianifolia* was dissolved in ethanol and then was added to then 10.0 g of silica gel. Then the solvent were removed using rotary evaporator until sample became sand-like.

Table 1: Ratio mixture of solvent used in Column Chromatography

Solvent	cyclohexane	EtOAc
	1	0
	7	3
	3	1
	1	1
	1	3
	3	7
	0	1

*300mL of solvent was used for all solvent ratios

2.7 Functional group detection

Combined fractions were analyzed using FTIR to detect their functional group. Samples were analyzed in solution form using DCM as solvent. The samples were put on sodium chloride palette and were analyzed directly. For background analysis, air was run as blank sample.

2.8 Preparative TLC

PTLC was used to further separate the compound that has been detected using CC. fractions that has good separation were used and dissolved in DCM. The samples then were put as a band on to glass coated silica gel and were developed using the same solvent as in analytical TLC. Then, a good band was scrapped off from the glass and were dissolved again using the same solvent used to develop it. Analytical TLC was used to determine how many spots were inside observed from the scrapped band. The solutions then were dried off using Nitrogen gas and the mass was obtained.

the scrapped band. The solutions then were dried off using Nitrogen gas and the mass was obtained.

2.9 Flavonoids detections

This methodology was adopted from Ahmad and Raji, 1993[1]

Flavonoids were tested using a simple test. Crude extract weighing 0.82 g were defatted using 10 mL hexane and repeated until solvent became clear. Then the crude was added with 20 mL 80% ethanol and was separated to 4 different test tubes labeled A, B, C and D respectively. Tube A is the control.

3.9.1 Wilstatter-Cyanidin test

For tube B, 0.5 mL of concentrated HCl was added followed by addition of 3-4 cm of magnesium tape and a color change was observed in ten minutes duration were recorded. Positive test showed that color changes from oranges to red (flavone), red to crimson (flavonol), crimson to magenta (flavanone) or green to blue. Color changes occurred usually in first 1-2 minutes after acid being added and the intensity of the color indicates the concentration of flavonoids.

2.9.2 Bate-Smith test

For tube C, 0.5mL of concentrated HCl was added. For positive result, red or purple appeared as soon as acid being added indicating chalcone or aurone.

2.9.3 Metcalf test

For tube D, 0.5mL of concentrated HCl was added and the tube were put inside a steam bath for 15 minutes and positive result is red color or purple appears in one hour duration indicating possibility of leucoanthocyanin.

2.10 Brine Shrimp Cytotoxicity Test

2.10.1 Hatching of brine shrimp

About two spatulas full of *Artemia salina* eggs was put into small clear container filled with seawater for hatching process. An aerator was used and placed inside the container to produce air bubble continuously. The brine shrimps were used after 48 hours for bioassay cytotoxicity test.

2.10.2 Testing procedure

Extract (50.0mg) was dissolved in 2 mL of ethanol solvent making solvent A. 1 mL of solvent A was taken and being added with 1mL of ethanol again making solvent B. From solutions A, 500 µL, 250 µL, 100 µL, 50 µL, and 5 µL and from solution B, 50 µL were taken and all samples taken were transferred to vials in triplicate. 5 mL seawater or sodium chloride solution (3.8 % in concentration) was added to each vial, resulting in final concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 100 µg/mL, 50 µg/mL and 10 µg/mL, and 10 *Artemia salina* were put into each vial. Amount of the survivors were counted within 1 hour, 3 hours, 6 hours, 12 hours and 24 hours contact. Then LC_{50} value was determined. Controls were made up with only seawater.

CHAPTER 3

3.0 RESULTS AND DISCUSSION

3.1 Extraction

The extraction of 100.0 g of dried sample of *Erechtites valerianifolia* with ethanol twice gave mass and percentage yield of the crude extract the plant as 8.242 g (8.242%)

3.2 Analytical TLC

All crude extract from different samples were subjected to TLC analysis using cyclohexane-ethyl acetate (3:1) as the developing solvents (Table 2). For *Erechtites valerianifolia* 14 spots were observed on the TLC plate under UV light (Figure 5).

Table 2: R_f values for components of the ethanol extract of *Erechtites valerianifolia*

Samples	Component	R_f Value
Erechtites valerianfolia	1	0.1012
	2	0.1392
	3	0.2532
	4	0.2785
	5*	0.3038
	6	0.3165
	7	0.3544
	8*	0.3924
	9	0.4177
	10*	0.4937
	11*	0.6329
	12	0.6709
	13*	0.7975
	14*	0.9114

* Major spot

Six clear and dark spots were observed which might indicate the presence of 6 major components. However, further fractionation and chromatography technique is needed to confirm this. The bright yellow spot is believe to be chalcone.[19, 20]

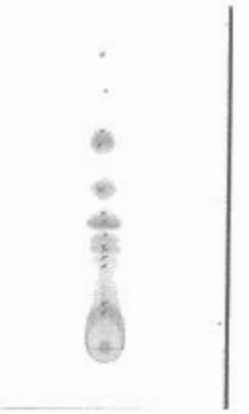

Figure 5: TLC of the crude extract of E. *valerianifolia*

3.3 2-Dimensional TLC

2.0 mg of crude extract (*Erechtites valerianifolia*) was use to run 2D TLC for flavonoid compounds identification. The developed spots were visualized under UV lamp with wavelength of 306 nm. 33 spots were observed which 1 spots appeared in florescence orange (R_f =0.8153) 1 spots appeared in florescence yellow (R_f=0.8662) and 31 spots appeared in florescence green.

3.4 Flavonoids detection

3.4.1 Wilstater –Cyanidin Test

The crude extract of *Erechtites valerianifolia* was to give color changes within 10 minutes. The color changed from green to orange and orange to red indicating the presence of flavone compound.

3.4.2 Bate-Smith test

For this test, color changes after acid addition was red or purple appears chalcone or aurone.

3.5 Fractionation

Only crude extract of *Erechtites valerianifolia* undergoes Column Chromatography. This is because of the extract shows more spots and best separation of spots on the TLC plate. In Column Chromatography, the crude extract of *Erechtites valerianifolia* was subjected to silica gel CC and eluted with eight solvents systems with increasing polarity, which were cyclohexane, cyclohexane-ethyl acetate (7:3), (3:1), (1:1), (1:3), (3:7) and ethyl acetate. Then, methanol solvent was used to flush out the remaining compounds left.

Through this fractionation procedure, 225 fractions were obtained which fractions 1-30 from cyclohexane, fractions 31-60 from cyclohexane-ethyl acetate (7:3), fractions 61-90 from cyclohexane-ethyl acetate (3:1), fractions 91-120 from hexane-ethyl acetate (1:1), fractions 121-150 from cyclohexane-ethyl acetate (1:3), fractions 151-180 from cyclohexane-ethyl acetate (3:7), fractions 181-200 from ethyl acetate and fractions 201-225 from methanol.

All the fractions were subjected to TLC analysis. The fractions were then combined based on the appearance of the spots and a total amount of 50 combined fractions were obtained. Tables 3 give the mass for each combined fraction obtained from CC

Table 3: Mass of the combined fractions obtained from column chromatography on crude extract of *Erechtites valerianofolia*.

Combined Fractions	Fractions	Mass (mg)
Ev01	1-20	2.8
Ev02	21	7.0
Ev03	22-23	62.0
Ev04	24-26	264.6
Ev05	27	210.3
Ev06	28-30	28.0
Ev07	31-35	18.0
Ev08	36-40	68.0
Ev09	41-42	16.0
Ev10	43-45	113.3
Ev11	46-50	21.0
Ev12	51-55	25.0
Ev13	56-60	16.9
Ev14	61-65	1.0
Ev15	66-70	159.6
Ev16	71-75	132.6
Ev17	76-80	37.0
Ev18	81-85	22.0
Ev19	86-90	40.0
Ev20	91-92	20.0
Ev21	93-94	42.0
Ev22	95-96	22.0
Ev23	97-98	21.0
Ev24	99-100	23.0
Ev25	101-105	10.0
Ev26	106-110	24.0
Ev27	111-115	31.0
Ev28	116-120	1.0
Ev29	121-125	1.0
Ev30	126-130	1.5
Ev31	131-135	1.3
Ev32	136-140	1.0
Ev33	141-145	3.0
Ev34	146-150	1.0
Ev35	151-155	1.3
Ev36	156-160	1.0
Ev37	161-165	1.0
Ev38	166-168	1.0