

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF
SOME MALAYSIAN HERBS

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TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF SOME
MALAYSIAN HERBS

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ABSTRACT

Herbs have drawn much attention of people worldwide, not only because of their economic value as food products, but also for their antioxidant compounds. Thus, this study was conducted to investigate the total phenolic content and antioxidant activities on three Malaysian herbs by using Soxhlet extraction method. The herbs were *Eugenia polyantha* (*Serai kayu*), *Euodia redlevi* (*Tenggek burung*) and *Limnocharis flava* (*Sudu itik*). The total phenolic content and antioxidant compounds were extracted using distilled water at three different extraction time (4, 8 and 12 hour). The extracts were then evaluated using Folin-Ciocalteu reagent for their total phenolic content and 2, 2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) assay for their antioxidant compounds. Further analyzed was done using High Performance Liquid Chromatography (HPLC) to further verifying the ascorbic acid existence in the extracts. It was found *L. flava* showed the highest content of total phenolic compounds (15.01 mg gallic acid equivalent (GAE) per 1 g of sample) with the antioxidant capacity standing at 20.42 mg ascorbic acid equivalent (AAE) per 1 g of sample after 8 hour extraction. While, *E. polyantha* showed the highest value of antioxidant capacity (21.19 mg AAE/g) with total phenolic content of 9.99 mg GAE/g. In HPLC analysis, it was found that *E. polyantha* and *L. flava* after 8 hour extraction contained 0.7625 and 0.9745 mg AAE/g of ascorbic acid respectively.

JUMLAH KANDUNGAN FENOLIK DAN AKTIVITI ANTIOKSIDAN DALAM BEBERAPA HERBA MALAYSIA

ABSTRAK

Herba telah menarik perhatian ramai orang di seluruh dunia, bukan sahaja kerana nilai ekonomi herba tersebut sebagai produk makanan, tetapi juga untuk sebatian antioksidan herba tersebut. Jadi, kajian ini telah dijalankan untuk menyiasat jumlah kandungan fenolik dan aktiviti antioksidan pada tiga herba Malaysia dengan menggunakan kaedah pengekstrak Soxhlet. Herba-herba tersebut adalah *Eugenia polyantha* (Serai kayu), *Euodia redlevi* (Tenggek burung) dan *Limnocharis flava* (Sudu itik). Jumlah kandungan fenolik dan sebatian antioksidan telah diekstrak menggunakan air pada tiga masa pengekstrakan yang berbeza (4, 8 dan 12 jam). Ekstrak kemudian dinilai menggunakan reagen Folin-Ciocalteu untuk jumlah kandungan fenolik dan kaedah 2, 2-Difenil-1-pikrilhidrazil hidrat (DPPH) untuk sebatian antioksidan. Analisis seterusnya telah dilakukan dengan menggunakan Kromatografi Cecair Prestasi Tinggi (HPLC) untuk mengesahkan kewujudan asid askorbik dalam ekstrak. *L. flava* telah didapati menunjukkan kandungan fenolik tertinggi (15.01 mg bersamaan asid gallic (GAE) setiap 1 g sampel) dengan kapasiti antioksidan 20.42 mg asid askorbik setara (AAE) untuk setiap 1 g sampel selepas 8 jam pengekstrakan. Sementara itu, *E. polyantha* menunjukkan nilai kapasiti antioksidan yang tertinggi (21.19 mg AAE/g) dengan jumlah kandungan fenolik 9.99 mg GAE/g. Dalam HPLC analisis, *E. polyantha* dan *L. flava* selepas 8 jam ekstrak masing-masing mengandungi 0.7625 dan 0.9745 mg AAE/g asid askorbik.

TABLE OF CONTENTS

| | PAGE |
|--|-------------|
| TITLE | i |
| SUPERVISOR'S DECLARATION | ii |
| STUDENT'S DECLARATION | iii |
| DEDICATION | iv |
| ACKNOWLEDGEMENT | v |
| ABSTRACT | vi |
| ABSTRAK | vii |
| TABLE OF CONTENTS | viii |
| LIST OF TABLES | xi |
| LIST OF FIGURES | xii |
| LIST OF SYMBOLS | xiii |
| LIST OF ABBREVIATIONS | xiv |
| LIST OF EQUATIONS | xvi |
| | |
| CHAPTER 1 INTRODUCTION | |
| 1.1 Research Background | 1 |
| 1.2 Problem Statement | 2 |
| 1.3 Research Objective | 3 |
| 1.4 Scopes of Study | 3 |
| 1.5 Significance of Study | 4 |
| | |
| CHAPTER 2 LITERATURE REVIEW | |
| 2.1 Herbs | 5 |
| 2.1.1 <i>Eugenia polyantha</i> (Serai kayu) | 6 |
| 2.1.2 <i>Euodia redlevi</i> (Tenggek burung) | 7 |
| 2.1.3 <i>Limnocharis flava</i> (Sudu itik) | 9 |
| 2.1.4 Findings from Previous Study | 10 |
| 2.2 Total Phenolic Contents | 12 |
| 2.3 Antioxidant | 13 |
| 2.3.1 Effect of Antioxidant to Free Radicals | 14 |

| | | |
|-----|---|----|
| 2.4 | Soxhlet Extraction Method | 17 |
| | 2.4.1 Soxhlet Extractor | 17 |
| | 2.4.2 The Usage and Benefits | 19 |
| | 2.4.3 Disadvantages of Soxhlet Extraction | 20 |
| 2.5 | Water Extraction | 20 |

CHAPTER 3 METHODOLOGY

| | | |
|-----|--|----|
| 3.1 | The Overall Methodology | 22 |
| 3.2 | Raw Materials | 23 |
| 3.3 | Chemicals | 23 |
| 3.4 | Equipments | 24 |
| | 3.4.1 Refrigerated Centrifuge | 24 |
| | 3.4.2 Freeze Dryer | 25 |
| | 3.4.3 Ultraviolet-Visible Spectrophotometer (UV-Vis) | 26 |
| | 3.4.4 High Performance Liquid Chromatography (HPLC) | 27 |
| 3.5 | Sample Preparation | 28 |
| 3.6 | Experimental Methods | 29 |
| 3.7 | Analysis Method | 31 |
| | 3.7.1 Preparation Standard Calibration Curve of Gallic Acid for Follin-Ciocalteu Assay | 32 |
| | 3.7.2 Determination of the Phenolic Content using Follin-Ciocalteu Assay | 32 |
| | 3.7.3 Preparation of Standard Calibration Curve of Ascorbic Acid for DPPH Assay | 33 |
| | 3.7.4 Determination of Antioxidant Activity using DPPH Assay | 33 |
| | 3.7.5 Determination of Ascorbic Acid Using High Performance Liquid Chromatography (HPLC) | 34 |

CHAPTER 4 RESULTS AND DISCUSSION

| | | |
|-----|---|----|
| 4.1 | Introduction | 36 |
| 4.2 | Moisture Content Determination | 36 |
| 4.3 | Determination of Total Phenolic Content in <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> Extracts | 38 |

| | | |
|--|--|----|
| 4.4 | Determination of Antioxidant Compounds from <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> Extracts | 40 |
| 4.5 | DPPH Free Radical Scavenging Activity | 43 |
| 4.6 | Relationship between Total Phenolic Content and Antioxidant Compounds | 46 |
| 4.7 | Determination of Ascorbic Acid in <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> | 49 |
| CHAPTER 5 CONCLUSION AND RECOMMENDATION | | |
| 5.1 | Conclusion | 51 |
| 5.2 | Recommendations | 52 |
| REFERENCES | | 54 |
| APPENDICES | | |
| | Appendix A | 60 |
| | Appendix B | 62 |
| | Appendix C | 64 |
| | Appendix D | 66 |

LIST OF TABLES

| | | PAGE |
|------------|--|------|
| Table 2.1 | Previous studies on the extraction of total phenolic content and antioxidant activity from <i>E. redlevi</i> , <i>E. polyantha</i> and <i>L. flava</i> | 11 |
| Table 4.1 | Determination of moisture content | 37 |
| Table 4.2 | The effect of extraction time on TPC from <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> extracts | 38 |
| Table 4.3 | The effect of extraction time on extraction of antioxidant compounds in <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> | 40 |
| Table 4.4 | Effect of extraction time on DPPH radical scavenging activity <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> | 44 |
| Table 4.5 | Total phenolic content and antioxidant compounds in <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> | 47 |
| Table 4.6 | Data analysis of the ascorbic acid of <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> extracts using HPLC analysis | 50 |
| Table C1.1 | Standard calibration curve of Gallic acid for Follin Ciocalteu Assay | 64 |
| Table C1.2 | Standard calibration curve of Ascorbic acid for Dpph assay | 65 |
| Table D.1 | Standard calibration curve of Ascorbic acid for determination of ascorbic acid by using HPLC | 66 |

LIST OF FIGURES

| | PAGE |
|------------|--|
| Figure 2.1 | <i>Eugenia polyantha</i> (Serai kayu) 7 |
| Figure 2.2 | <i>Euodia redlevi</i> (Tenggek burung) 9 |
| Figure 2.3 | <i>Limnocharis flava</i> (Sudu itik) 10 |
| Figure 2.4 | The structure of phenol (C ₆ H ₅ OH) 12 |
| Figure 2.5 | Action of antioxidant inside the body 14 |
| Figure 2.6 | The formation of free radicals 16 |
| Figure 2.7 | Soxhlet Extractor 19 |
| Figure 3.1 | The pictures of herbs chosen (a) <i>E. redlevi</i> (b) <i>L. flava</i> (c) <i>E. polyantha</i> 23 |
| Figure 3.2 | Refrigerated centrifuged 25 |
| Figure 3.3 | Freeze dryer 26 |
| Figure 3.4 | Ultraviolet-Visible spectrophotometer (UV-Vis) 27 |
| Figure 3.5 | High Performance Liquid Chromatography (HPLC) 28 |
| Figure 3.6 | Flowchart of sample preparation 30 |
| Figure 3.7 | Flow chart of experimental study with different extraction and different samples 31 |
| Figure 4.1 | Effect of extraction time on phenolic content in <i>E.</i> <i>polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> 38 |
| Figure 4.2 | Effect of extraction time on antioxidant compounds <i>E.</i> <i>polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> 41 |
| Figure 4.3 | DPPH free radical scavenging activity of <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> 44 |
| Figure 4.4 | The correlation between phenolic content and antioxidant compounds of <i>E. polyantha</i> , <i>E. redlevi</i> and <i>L. flava</i> 47 |
| Figure A.1 | Gantt chart for Undergraduate Research Project I 60 |
| Figure A.2 | Gantt chart for Undergraduate Research Project II 61 |
| Figure B.1 | <i>E. polyantha</i> powder 62 |
| Figure B.2 | <i>L. flava</i> extract 62 |
| Figure B.3 | Soxhlet extraction 63 |

| | | |
|-------------|--|----|
| Figure B.4 | Follin-Ciocalteu Assay | 63 |
| Figure C1.1 | Graph of standard calibration curves of Gallic acid for Follin Ciocalteu Assay | 64 |
| Figure C1.2 | Graph of standard calibration curves of Ascorbic acid for DPPH Assay | 65 |
| Figure D.1 | Graph of standard calibration curves of Ascorbic acid for determination of ascorbic acid by using HPLC | 66 |
| Figure D.2 | HPLC graph for <i>Eugenia polyantha</i> | 68 |
| Figure D.3 | HPLC graph for <i>Limnocharis flava</i> | 69 |

LIST OF SYMBOLS

| | |
|------------------------|---------------------------|
| % | Percent |
| ϵ | Dielectric constant |
| $^{\circ}\text{C}$ | Degree celcius |
| g | Gram |
| g/L | Gram over liter |
| h | Hour |
| H_2O_2 | Hydrogen peroxide |
| L | Liter |
| M | Molar |
| ml | Milliliter |
| ml/min | Milliliter over minute |
| mg/ml | Milligram over milliliter |
| mm | Millimeter |
| nm | Nanometer |
| O_2 | Oxygen |
| ppm | Part per million |
| w/w | Weight over weight |
| w/v | Weight over volume |
| $\mu\text{g/ml}$ | Microgram over milliliter |

LIST OF ABBREVIATIONS

| | |
|--------|--|
| AAE | Ascorbic acid equivalent |
| BHA | Butylated hydroxyanisole |
| BHT | Butylated hydroxytoluene |
| DPPH | 2, 2-Diphenyl-1-picrylhydrazyl hydrate |
| GAE | Gallic acid equivalent |
| HPLC | High performance liquid chromatography |
| ROS | Reactive oxygen species |
| PG | Propyl gallate |
| TAC | Total antioxidant capacity |
| TAE | Tannic acid equivalent |
| TPC | Total phenolic content |
| TBHQ | Tertbutylhydroquinone |
| UV-Vis | Ultra-violet visible spectrophotometer |

LIST OF EQUATIONS

| | PAGE |
|--|-------------|
| Equation 3.1 Percentage inhibition (%) formula | 35 |
| Equation 4.1 Moisture content (%) formula | 38 |
| Equation 4.2 Ascorbic acid content formula | 52 |

CHAPTER 1

INTRODUCTION

1.1 Research Background

Malaysia has abundant herbs to be explored. According to Forestry Research Institute (FRIM), there are about 6,000 species of herbs in Malaysia that can be developed and used as beauty and food supplement products. FRIM also had studied more than 20 species of medicinal and aromatic plants. Herbs are being used by about 80% of the world population especially in the developing countries for primary health care (Jain *et al.*, 2011). Interest has been expressed in herbs in recent years because herbs are belief as natural sources of antioxidants. Antioxidant is the bioactive compounds from plant extracts which have been discovered to exhibit antioxidant activity.

The different antioxidant components found in herbs provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and heart disease (Pandi and Rizvi, 2009). Nowadays, herbs have been used for many purposes including medicine, nutrition, flavorings,

beverages, and industry. The antioxidative effect is mainly due to phenolic components such as simple phenols, flavonoid and phenolic acids. The antioxidant activity of phenolic compounds is mainly due to their redox properties. The phenolic content present in the herbs have anti-mutagenic, anti-cancerogenic properties, cardioprotective, anti-inflammatory and antimicrobial activity (Stanojevic *et al.*, 2009).

The principle function of antioxidants is in delaying or preventing degenerative diseases caused by oxidative damage of living cell components caused by free radicals (Qader *et al.*, 2011). Free radicals contribute to the development of many diseases such as hemorrhagic shock, arthritis, ageing, atherosclerosis, ischemia, Alzheimer and Parkinson's disease, gastrointestinal disorders, and tumor promotion. Free radicals molecules are produced during the process of breaks down food. Besides that, free radical also can be produced when we expose to radiation and tobacco smoke. The examples for reactive free radicals are superoxide, hydroxyl, peroxy and alkoxy. To prevent our body from effect of free radical much research has been done to investigate the effects of antioxidants to human health.

1.2 Problem Statement

Some Malaysian herbs have high quantity of antioxidant compounds. However, some of the herbs still lack in scientific data regarding the actual potential of these herbs on antioxidant activity. Function of antioxidants is to prevent many several diseases like cancer and aging. Natural antioxidant from herbs is safer for human body compared to the synthetic antioxidant.

This research was conducted to explore phenolic content and antioxidant in three herbs. The herbs have been chosen because of their potential source of antioxidant. Besides that, it is because these herbs are not very common in our community. Antioxidants in these three herbs are very useful for human health. It is free from side effects compared to synthetic antioxidants.

1.3 Research Objectives

The aim of this study is to investigate the total phenolic content and antioxidant capacity from some Malaysia herbs. The selected herbs are *Eugenia polyantha* (*Serai kayu*), *Euodia redlevi* (*Tenggek burung*) and *Limnocharis flava* (*Sudu itik*).

1.4 Scopes of Study

In order to achieve the objectives, there are few scopes of work that have been identified as follows:

- i. Extracting the total phenolic content of herbs by using Soxhlet extraction method. Herbs were extracted at three different times (4, 8, and 12 hour) at 100°C.

- ii. Determining the total phenolic content and antioxidant capacity of herbal water extracts. Total phenolic content and antioxidant activity were determined using the Follin-Ciocalteu method and 2, 2-Dipheneyl-1-Picrylhydrazyl hydrate (DPPH) assay respectively.
- iii. Demonstrating a possible relationship between total phenolic content and antioxidant compounds from herbal water extracts.
- iv. Identifying the ascorbic acid content from herbal water extracts using High Performance Liquid Chromatography (HPLC).

1.5 Significant of Study

Eugenia polyantha (Serai kayu), *Euodia redlevi* (Tenggek burung) and *Limnocharis flava* (Sudu itik) are promising source of natural antioxidant. These herbs have good potential to be used as natural antioxidant. With high level of antioxidant content, these herbs are potential as a good alternative for natural antioxidant. Antioxidant that contain in these herbs will give extra value to herbs itself. This will give benefits to farmer by plant it in large scale. It is expected that the information and knowledge gained from this research studies will increase the awareness of using natural antioxidant besides synthetic antioxidant.

CHAPTER 2

LITERATURE REVIEW

2.1 Herbs

The natural occurring antioxidant is focused more on edible plants, especially herbs (Faujan *et al.*, 2009). Herbs were used long ago by people around the world. Herbs are any plant used for flavoring, food, medicine or perfume. There are numerous different herbs, each one having its own unique components. The uses of natural antioxidants from herbs extracts have experience growing interest due to some human health professionals and consumer's concern about the safety of synthetic antioxidants in foods (Sun and Ho, 2005; Suhaj, 2006). Herbs are an excellent source of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids which have been reported to show good antioxidant activity (Zheng and Wang, 2001). Generally, Malaysian foods are rich in herbs including *E. polyantha*, *E. redlevi* and *L. flava*.

2.1.1 *Eugenia polyantha* (Serai kayu)

Eugenia polyantha or a synonym *Syzygium polyanthum* is a deciduous tropical tree belonging to the Myrtaceae family. The previous studies on Myrtaceae family showed their plants are great potential source of antioxidants due to many detected phenolics and flavonoids (Lee and Intan, 2012). Most species are evergreen trees and generally known as *Serai kayu*, *Daun salam* (bay leaf), *Samak kelat* and *Daun salam manting*. *E. polyantha* mostly grown in the forest, but they may be planted in the garden. It height is about 25 meters, have straight root, round trunk and smooth surface. Besides that, *E. polyantha* has fragrant smell and has astringent to the taste. According to that, the leaves of this plant are usually added in local dishes for its pleasant aroma (Agus and Agustin, 2008).

E. polyantha has a lot of chemical properties that useful in medical, even as basic materials in dentistry. *E. polyantha* has some pharmacologic activities that are useful in dentistry. The previous studies showed that tanine, flavonoid, essential oil, including citric acid and eugenol are present in *E. polyantha*. Tanine is one of active matters of *E. polyantha* and part of phenol group that can inhibit the growth of bacteria by precipitation and denaturation of bacteria protein. Flavonoid in *E. polyantha* has an anti-inflammatory effect and can support vascular wall, therefore the bleeding can be stopped. While, essential oil have antiseptic and antioxidant activity. Essential oil also has activity to inhibit the growth of some bacteria and fungi. Overall, the chemical properties of *E. polyantha* have analgesic, antibacterial and anti-inflammatory effect (Agus and Agustin, 2008).

E. polyantha can be used not only as spices for cooking purposes, but it can be used as medicine. *E. polyantha* has been known since long time ago as a species

that can be used for therapy. The use of *E. polyantha* has been developed medically, as an alternative medical plant. Empirically, *E. polyantha* can be used for hypertension, drunks and skin diseases (Raden *et al.*, 2009). The plant also has other benefits such as diuretic and analgesic effect. Its roots and fruits extract have the ability to neutralize overdoses of alcohol consumption. Besides those two utilities, *E. polyantha* extract is usually used to stop diarrhea, gastritis, diabetes mellitus, itchy, astringent and scabies. *E. polyantha* can also be used to treat patients with high uric acid (Agus and Agustin, 2008). *E. polyantha* plants can be used as a safe, reliable and economical natural source in pharmaceutical and food industry.



Figure 2.1 *Eugenia polyantha* (*Serai kayu*)

2.1.2 *Euodia redlevi* (*Tenggek burung*)

Euodia redlevi is one of the most common medicinal herbs that are widely distributed in many areas of Peninsular Malaysia and also in several other Asian countries. Besides that, *E. redlevi* or locally known as *Tenggek burung* which

belongs to family Rutaceae is a popular traditional fresh vegetable among the Malaysian community (Faridah *et al.*, 2010). People love to eat this leaves as *ulam* and goes well with *sambal belacan*, *tempoyak* or *sambal kelapa*. Nowadays, *E. redlevi* is one of the alternative herbal resources which have a great potential to be marketed worldwide. From the findings, it is reported that *E. redlevi* had a slightly bitter taste, crunchy young leaves, pungent and lemon-lime aroma. In terms of its physical characteristics, it is said that *E. redlevi* has trifoliate, green, thick, broad leaves and has small white and greenish flowers (Karim *et al.*, 2011).

Previous study on *E. redlevi* yielded interesting secondary metabolites including flavonoids, alkaloids, coumarins, and acetophenones (Parsons *et al.*, 1994). Different parts of *E. redlevi* have been used traditionally as natural remedy for fever, emmenagogue, stomach ache, and rheumatism as well as treatment of wounds and itches. In addition there have been many other usages of the herb. For example, to prevent premature ejaculation, as an aphrodisiac and for its blood pressure lowering effects (Faridah *et al.*, 2010). The almost ripe fruit of this herb are used in Korean folk medicine as an analgesic, antiemetic, astringent and also as hypertensive agent. Besides, they also reported other uses of this species as an antipyretic, anti-inflammatory, reduces fatigue, improve blood circulation and relieve body stamina. It also acts as an aphrodisiac especially for men. Besides, this herb also contains a lot of antioxidants which is good for cancer (Karim *et al.*, 2011).



Figure 2.2 *Euodia redlevi* (*Tenggek burung*)

2.1.3 *Sudu itik*

Limnocharis flava (*Sudu itik*) from family Limnocharitaceae is a soft stem plant. *L. flava* reproduces mainly by the production of seeds. This herb can be found in the bush and has physical properties such as thin and green leaves. *L. Flava* commonly used as a vegetable in dishes either cooked or raw. The herb contains relatively high levels of Ca, Fe and β -carotene, which are minerals that are frequently insufficient in the diets of women in low-income countries (Saupi *et al.*, 2009).

Besides that, *L. Flava* provide good sources of other minerals such as potassium, zinc, magnesium and copper. Other than servings as food, the nutritive values contained in *L. Flava* are protein, fat, carbohydrate, crude fiber, vitamin C and calorific. These properties are able to improve appetite, digestive system and increase availability of nutrient in body to maintain our health (Abhilash, 2003). *L. Flava* is used to treat ear pain, eye, tumors, boils, swelling, asthma, malaria,

hemorrhoids, gum teeth and hypertension. For the use of ear pain and boils, squeezed the leaves until soft and put the juice into the ear and repeat until relieved.



Figure 2.3 *Limnocharis flava* (*Sudu itik*)

2.1.4 Findings from Previous Study

Some researchers have studied on the extraction of total phenolic content and antioxidant activity from *E. Redlevi* and *E. Polyantha*. However, there was lack research being done on *E. Flava*. Based on previous study (Rafat *et al.*, 2010; Shaidat *et al.*, 2011), determination of phenolic content and antioxidant compounds on *E. Redlevi* has been done by solvent extraction. Rafat *et al.* (2010), was extract *E. Redlevi* at 27°C for 72 hours by using ethanol as a solvent and reported 65.33 ± 1.91 mg GAE/g and 53.15 mg AAE/g for value of TPC and TAC. While, Shaida *et al.*

(2011) was extract *E. Redlevi* at 27°C for 1 hour by using distilled water as a solvent and gave the value 3.4 ± 0.5 mg GAE/g and 0.3 ± 0.1 mg GAE/g for TPC and TAC.

The presence of TPC and TAC in *E. Polyantha* was approved by Wong *et al.* (2006) and Perumal *et al.* (2012). The extraction was done by solvent extraction by using deionized water at 27°C for 1 hour and methanol at 27°C for 6 days. Studied done by Perumal *et al.* (2012) by using methanol as a solvent gave the higher value for TPC (333.75 ± 1.92 mg GAE/g). Their findings can be summarized in Table 2.1.

Table 2.1 Previous study on the extraction of total phenolic content and antioxidant activity from *E. redlevi* and *E. polyantha*.

| Type of herbs | Extraction method | Extraction parameter | Total phenolic contents (TPC) | Total antioxidant capacity (TAC) | Reference |
|--|--------------------|--|-------------------------------|----------------------------------|------------------------------|
| <i>Euodia redlevi</i> (Tenggek burung) | Solvent extraction | Solvent: ethanol T: 27°C t: 3 days | 65.33 ± 1.91 mg GAE/g | 53.15 ± 1.02 mg AAE/g | Rafat <i>et al.</i> (2010) |
| | Solvent extraction | Solvent: distilled water T: 27°C t: 1 hour | 3.4 ± 0.5 mg GAE/g | 0.3 ± 0.1 mg GAE/g | Shaida <i>et al.</i> (2011) |
| <i>Eugenia polyantha</i> (Serai kayu) | Solvent extraction | Solvent: deionized water T: 27°C t: 1 hour | 12 mg GAE/g | 120 μ mol TAE/g | Wong <i>et al.</i> (2006) |
| | Solvent extraction | Solvent: methanol T: 27°C t: 6 days | 333.75 ± 1.92 mg GAE/g | 20.90 ± 0.26 μ g/ml | Perumal <i>et al.</i> (2012) |
| <i>Limnocharis flava</i> (Sudu itik) | Nil | Nil | Nil | Nil | Nil |