**Modern Agricultural Science and Technology, ISSN 2375-9402, USA**
**December 2017, Volume 3, No. 5-6, pp. 18-21**
**Doi: 10.15341/mast(2375-9402)/03.03.2017/004**
**Academic Star Publishing Company, 2017**
**www.academicstar.us**

# Phytochemicals Screening and Antioxidant Activity of Three Different Solvent Extracts of *Euodia Redleyi* Leaves

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**Abstract:** This study was conducted to identify the phytochemicals screening and antioxidant activity of three different solvent extracts of Euodia Redleyi leaves**.** The sample was collected at Pahang, Malaysia. Hexane, dicholoromethane (DCM) and methanol extracts of the leaves were used in this study. The established conventional methods were used for quantify the total phenol, flavonoids contents and antioxidant activity. Phytochemical screening test determined the presence of flavonoids, tannin, saponin, terpenoid and phenolic content. Total antioxidant activities were determined by DPPH method and Total phenolic content (TPC) were determined by Follin-Ciocalteau method. A screening test for E.redleyi showed the presence of all phytochemical but absence of saponin. There was a significant difference between hexane, DCM and methanol plant extracts; hexane and DCM extracts were more prominent in antioxidant activity but methanol extraction was more prominent in TPC. Since E. redleyi exhibited high antioxidant and TPC; it can be used as a natural antioxidant. Further study needed to identify the exact active compound underlying this high antioxidant activity.

**Key words:** euodiaredleyi, solvent, phytochemicals screening, antioxidant activity, DPPH ass

## 1. Introduction

Plants and herbal extracts are considered as important materials in modern medicine, due to their phytochemical and medicinal contents in their natural form. Malaysian herb are used as side dish as also known as a raw vegetable. They also have been receiving special attention because of their history in folk medicinal uses either for preventative or even curative purposes. Not many research have been conduct for this selected herbs [1]. Thus two types of Malaysian herb were selected to determine their phytochemicals and antioxidant activities**:** *Euodiaredleyi* locally name *tenggekburung* and usually used traditionally during confinement period and it is very helpful to lower the blood pressure and blood circulation. Plant extracts and their constituents can act as a natural source of antioxidants have been extensively reviewed. An antioxidant refer as any substance that, when present at low concentrations significantly delays or prevents oxidation of cell content like proteins, lipids, carbohydrates and DNA [2]. Over the year a large number of experiments have been carried out concerning the antioxidant activity of several plant extracts and powders [3]. Therefore, the research of natural product like natural antioxidants from plant and herbal extract not only healthier and safer than synthetic one but it also will be more acceptable among modern consumers.

### *2. Materials and Methods*

#### *2.1 Methodology*

*Euodiaredleyi* leaves was collected at Pahang, Malaysia. The sample have been sliced and stripped are dried in an oven at 60°C for 24 hours. The dried

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samples were ground to powder form and kept in airtight plastic containers before extraction process.

*2.2 Extraction of Plant Materials*

Powdered samples were extracted in Soxhlet extractors using hexane, dichlorometane (DCM) and methanol as a solvent. The samples are inserted in a thimble and the apparatus was set up. Then, 250 ml solvent is filled in the round bottom flask and reflux until become colourless. Extraction started with hexane and followed by dichloromethane and methanol. The extracts were concentrated using Heidolph Rotary Evaporator with vacuum pump with the water bath set at temperature 40°C.

*2.3 Phytochemicals Screening Test*

2.3.1 Evaluation of Phytochemicals Screening Test

The crude extracts of samples were analyzed for the presence of phytochemical compounds which is flavonoid, phenolic content, saponins, tannins and terpenoids.

2.3.2 Sample Preparation

0.05 g of samples were weighed and each sample is diluted with 20 ml of it solvents (hexane, dichloromethane and methanol). The sample is stirred until fully dissolved in the solvents.

2.3.3 Test for Flavonoids

A few drop of sample was dropped in the test tube. 5 ml of sodium hydroxide and 1ml of nitric acid is added. The test tube was shaken and yellow coloration disappear indicates the presence of flavonoids.

2.3.4 Test for Tannins

A few drops of the crude extract sample are placed in a test tube. About 2 ml of distilled water is added and the mixture shaken and then placed in a water bath for 5 minutes at 80°C to 100°C. Next 2 or 3 drops of 0.1% iron (III) chloride was added and observed for brownish green or a blue-black colouration [1].

2.3.5 Test for Saponins

A few drops of the crude extract sample are placed in the test tube. About 5 ml of distilled water was added and shaken vigorously. The formation of stable bubble froth indicates the presences of saponins [1].

2.3.6 Test for Terpenoids (Sakowski Test)

A few drops of the crude extract sample was added 2 ml of chloroform. Concentrated $H_2SO_4$ (3 ml) was carefully added to form a layer. A reddish brown colouration indicates the presence of terpenoids [1].

2.3.7 Test for Phenolic Content

A few drops of the crude extract sample are dropped into a test tube. About 2 ml of distilled water were added followed by 2 or 3 drops of 10% iron (III) chloride solution is added. The presence of green or blue colour indicates the presence of phenolic compounds [4].

*2.4 Determination of Antioxidant.*

2.4.1 Preparation of Standard Sample and Solution

The antioxidant activity of plant extracts and the standard was assessed on the basic of free radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) which is free radical activity by modified method [5]. The dilute working solutions of the test extracts were prepared in methanolic solution to give the concentration 1mg/ml.

2.4.2 Preparation of DPPH solution

The stock of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was prepared. The 0.01 g of 1,1-diphenyl-2-picrylhydrazyl (DPPH) is diluted in 250 ml methanol. It was protected from light by covering the volumetric flask with aluminium foil.

2.4.3 DPPH Scavenging Activity

DPPH concentration is reduced by the existence of an antioxidant at 517 nm and the absorption gradually disappears with time. The Unico spectrophotometer was used to determine the antioxidant activity. From the dilution 0.02 ml of sample is added with 0.8 ml of methanol and 2 ml of DPPH solution. The mixture was shake using vortex for 30 seconds and leave 30 minutes in dark condition described by Molyneux et al. (2004) [6]. Extract concentration providing 50% inhibition

(IC50) calculate from the graph plotted of inhibition percentage against extract concentrations.

*2.5 Determination of Total Phenolic Content (TPC)*

2.5.1 Principle of Method

Total phenolic content were determined by Follin-Ciocalteau method [7], based on complex formation of molybdenum-tungsten bleu. The samples were allowed to react with Follin-Ciocalteau's reagent and sodium carbonate solution. The phenolic contents were estimated using a standard curve of gallic acid and expressed as mg of gallic acid equivalents (GAE) g of extract.

2.5.2 Sample Analysis

Total phenolic content were measured using the modified Folin-Ciocalteau method [8]. Hexane, dichloromethane and methanol crude extract of samples was weighed 0.01 g and dissolved with it solvent up to 10 ml solution. 1 ml sample of extract was transferred into a test tube and 2 ml of 50% Folin-Ciocalteu reagent was added and mixed. The mixture was allowed to stand at room temperature for 5 min. Then, 4 ml of 5% $Na_2CO_3$ was added to the mixture and mixed gently. After standing at room temperature for an hour, the absorbance was read at 765 nm using UV/Vis Spectrophotometer (Shimadzu UV-1650 PC spectrometer). The absorbance values were compared with gallic acid calibration standards in the range between 0.2-1.0 μg/ml.

## 3. Results and Discussions

*3.1 Yield of Samples.*

*Euodiaredleyi* leaves show high extraction in methanol (13.96±0.20%) than hexane (10.66±0.25%) and dichloromethane (5.06±0.35%). Next, *C. asiatica* leaves show high result of crude extraction using methanol (16.60±0.23%) than hexane (10.55±0.19%) and dichloromethane (1.09±0.30%). The results were summarized in the following Table 1.

**Table 1 The percentage of yield extraction of *Euodiaredleyi* in different solvents**

| Samples | Percentage of crude extract (%) | | |
|---|---|---|---|
| | Hexane | Dichloromethane | Methanol |
| *E. redleyi* | 10.66±0.25 | 5.06±0.35 | 13.96±0.20 |

*3.2 Phytochemical Screening of Plant Materials*

Different phytochemicals test were used to identify the phytochemical that are present in the samples. The results of the tests are shown in Table 2.

The phytochemical screening of the plants studied showed the presence of flavonoids, terpenoids, saponins and phenolic compound in the samples but *E. redleyi* extract showed the negative for the presence of saponin.

**Table 2 Phytochemical Screening of difference solvent extracts of *E.redleyi*.**

| Samples | Flavonoids | | | Taninns | | | Saponins | | | Terpenoids | | | Phenolic compound | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H | D | M | H | D | M | H | D | M | H | D | M | H | D | M |
| *Euodiaredleyi* | + | ++ | +++ | - | + | ++ | - | - | - | + | - | - | - | ++ | +++ |

H: Hexane, D: Dichloromethane, M: Methanol, `+++' sign indicates the compunds strongly present,`++' sign indicates moderately present, `+' sign indicates weak present and `-' sign indicates the compounds are absent.

*3.3 TPC Assay*

A linear calibration curve of Gallic acid with correlation coefficient ($R^2$) value of 0.9772 was obtained (not shown). TPC of the plants leave extracts measured using the GAE equation of y = 0.4855x+0.0225 ($R^2$ = 0.997), whereby y = absorbance at 765 nm and x = concentration of total phenolic compounds in mg per mL of the extract. The total phenol contents of extract are shown in Table 3. The amount of total phenolic varied from 0.27±0.15 mg GAE/mL to 3.19±0.15 mg GA/mL The results obtained in *E. redleyi* show that the total phenolic in methanolic extracts had the highest TPC (3.19±0.15 mg/mL), followed by *DCM extracts* (1.87±0.09

mg/mL and finally hexane extracts (1.22±0.25 mg/mL).

*3.4 DPPH Radical Scavenging Activity Assay*

The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in visible region. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract.

$IC_{50}$ value was determined from plotted graph of scavenging activity against the different concentrations of samples. The scavenging activity was indicated by the percentage of DPPH reduction after 30 min of reaction. The measurements were duplicate and their scavenging effects were calculated based on the percentage of DPPH scavenged [9, 10]. The less $IC_{50}$ value, the more powerful of the antioxidant. The obtained results are summarized in Table 4.

**Table 3 Total phenolic contents (mg/ml) value of difference solvent extracts of *E.redleyi.***

| EXTRACT | Phenol contents (mg/ml) |
|---|---|
| Hexane | 1.22 ±0.25a |
| DCM | 1.87±0.09a |
| Methanol | 3.19±0.15b |

**Table 4 $IC_{50}$ (mg/ml) value of difference solvent extracts of *E.redleyi*.**

| EXTRACT | DPPH $IC_{50}$ (mg/ml) |
|---|---|
| Hexane | 0.28±0.15a |
| DCM | 0.24±0.09a |
| Methanol | 0.36±0.25b |

## 4. Conclusion

Based on the present result and previous study we can conclude that the extracting solvent influenced the antioxidant and TPC activity of *Euodia Redleyi* leaves. Obviously, there was a significant difference between hexane, DCM and methanol plant extracts; hexane and DCM extracts were more prominent in antioxidant activity but methanol extraction was more prominent in TPC. Since *E. redleyi* exhibited high antioxidant and TPC; it can be used as a natural antioxidant. Further study needed to identify the exact active compound underlying this high antioxidant activity.

## Acknowledgements

The authors express gratitude to Department of Food Technology, Polytechnic of Sultan Haji Ahmad Shah and JPP to the grant FRGS/1/2014/STWN03/JPP/03/02 for the financial support.

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