

Phytochemicals Screening and Antioxidant Activities of Aqueous Leaves Extract of *Euodia ridleyi* and *Colubrina asiatica*

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Abstract: This study was conducted to identify the presence of phenol, flavonoids and antioxidants resulted from the aqueous extracts of the leaves of *Euodia ridleyi* (Tenggek burung) and *Colubrina asiatica* (Peria pantai). The established conventional methods were used for quantify the total phenol, flavonoids contents and antioxidant activity. Phytochemicals screening were conducted to determine the presence of flavonoids, tannin, saponin, terpenoid and phenolic content whilst DPPH method were used in determining total antioxidant activities. The positive result obtained from a screening test for both plants as it showed the presence of flavonoids, tannin, saponin, terpenoid and phenolic content. The analysis of total phenolic showed *E. ridleyi* contains 44.56 ± 0.35 mgGAE/g and 20.97 ± 0.53 mgGAE/g for *C. asiatica*. The result of total flavonoid showed *C. asiatica* has contained 1.51 ± 0.09 mgQE/g and 0.14 ± 0.03 mgQE/g for *E. ridleyi*. Concentrations of the plant extracts required for 50% inhibition of DPPH radical scavenging effect (IC_{50}) were recorded as 5.04 ± 2.17 for *E. ridleyi* and 5.85 ± 0.17 for *C. asiatica*. Thus, this study is useful in developing plant based antioxidant, which expected to be superior to synthetic antioxidant especially in the development of drugs to treat various diseases.

Keywords: *Euodia ridleyi*, *Colubrina asiatica*, phytochemicals screening, antioxidant activity, DPPH assay

1. Introduction

Medicinal plants have been receiving special attention not only due to their history in folk medicinal but also play an important role in the modern medicine. The herbs have been used as traditional treatments either for preventative or even curative purposes and in addition, a valuable natural resources as potentially safe drugs [Hassawi and Kharm, 2006; Bhat. et al., 2009]. Plants and herbal extracts are considered as important materials in modern medicine, due to their phytochemical and medicinal contents in natural form. According to Rasha Saad et al. 2014, natural drugs is usually used to cure some ailments which may not be treated by conventional medicine.

Over the years, the study of antioxidants as substances that used to fight against the free radicals has attracted the concerns of the public and researchers (Rasha Saad et al. 2014). Yet, as not many research have been conduct for selected herbs (Ayoola et.al, 2008), therefore two types of herbs, namely *Euodia ridleyi* and *Colubrina asiatica* were selected in determining their phytochemicals and antioxidant activities through this study.

Euodia ridleyi locally name *tenggek burung* is traditionally used during confinement period and very helpful in lowering high blood pressure and blood circulation. *Colubrina asiatica* or *peria pantai* is also called lather leaf because of its ability to produce lather in water. *C. asiatica* is eaten with rice after being half-boiled and it is claimed to improve digestion. Traditionally, *C. asiatica* used in the treatment of headache and body aches, fever and pain reliever, improve digestion, stabilize blood pressure, help control diabetes, as an abortifacient as well as a piscicide and migraine remedy (Saidin,2010).

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 (Vivek & Suraj, 2006). Currently, research on free radical confirms that essential role play by rich food in antioxidant in the prevention of cardiovascular diseases and cancer (Valko et al, 2007) and (Ljubuncic et al, 2005). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) widely used in food industries, however have been examined for possible toxicity. Hence, more studies on natural antioxidants derived from plants to replace the synthetic antioxidants. Therefore, the application of natural product like natural antioxidants as food additives not only healthier and safer than synthetic one but also will be more acceptable among modern consumers.

2. Materials and Methods

2.1 Plant Material Preparation

The leaves of *Euodia ridleyi* (tenggek burung) and *Colubrina asiatica* (peria pantai) were obtained fresh from Kampung Pandan 2 and Kampung Padang, Kuantan Pahang. These specimens were then sent to the botanist for confirmatory identification. All samples were washed using tap water and the damaged and disease portions were removed. All chemicals and solvents used were of analytical grade unless otherwise specified.

2.2 Sample Collection and Extraction

The plant sample was cut into small pieces and the leaves were dried in a dryer (Protech) at 40°C for two weeks, grind (Qingdao Dahua Double Circle), labeled and stored in airtight container for further used. An amount of 50g of ground samples was extracted using 250 mL deionized water.

The mixture was allowed to stand for 24 hour at 60°C. The aqueous extract was obtained by filter the mixture through Whatman No. 1 filter paper and used for analysis without

further treatment. The extract was kept in Biomedical refrigerator (CHRIST, BETA 1-8 LD plus) (at -20°C) and freeze dried (Fiocchetti, Scientific Refrigerator) for 8 days.

Percentage yield of extraction (%) = $\frac{\text{weight of crude extract in beaker after dried(g)} - \text{weight of blank beaker (g)}}{\text{Weight of sample in thimble before dried (g)}} \times 100\%$

2.3 Evaluation of Phytochemicals Screening Test

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

Sample Preparation

0.05g of sample was weighed using digital balance in 100ml beaker. Each sample is then diluted with 20ml with distilled water.

Test for Flavonoids

A few drop of sample was dropped in the test tube. 5ml of sodium hydroxide and 1ml of nitric acid was added into the tube. The test tube was shaken until the yellow coloration disappear, indicates the presence of flavonoids (Ayoola et al, 2008).

Test for Tannins

A few drops of the sample crude extract are placed in a test tube. 2ml of distilled water is added. The mixture was shaken before placing into water bath for 5 minutes at 80°C to 100°C. after that, two or three drops of 0.1% iron (III) chloride was added and observed for brownish green or a blue-black colouration. (Ayoola et al, 2008).

Test for Saponins

A few drops of the sample crude extract are placed in the test tube. About 5ml of distilled water was added and shaken vigorously. The formation of stable bubble froth indicates the presences of saponins. (Ayoola et al, 2008).

Test for Terpenoids (Sakowski Test)

A few drops of the sample crude extract are added with 2 ml of chloroform. Carefully, follow with 3ml concentrated H₂SO₄. A reddish brown colouration indicates the presence of terpenoids. (Ayoola et al, 2008).

Test for Phenolic Content

A few drops of the sample crude extract are dropped into a test tube. About 2ml of distilled water were added followed by 2 or 3 drops of 10% iron (III) chloride solution. The presence of green or blue color indicates the presence of phenolic compounds. (Sabri et al, 2012)

2.4 Determination of Total Flavonoid Content (TFC)

About 0.01g sample were diluted with distilled water to obtain 0.01g/ml of the sample concentration. Then, 1.0ml sample was mixed with 4ml of distilled water and subsequently with 0.3ml of a NaNO₃ solution (10%). After 5 minutes, 0.3ml AlCl₃ solution (10%) was added, followed with 2.0ml of NaOH solution (1%) to the mixture. The sample absorbance was measured at 510 nm with a UV-Visible spectrophotometer. Distilled water was used as a blank. The total flavonoid concentration in water extract was

then calculated from quercetin hydrate (Qu) calibration curve (0.1-0.5 mg/ml) and expressed as quercetin equivalents (Qu)/g of dry extract. Measurements were done in triplicates.

2.5 Determination of Total Phenolic Content (TPC)

Principle of method

Total phenolic content were determined by Follin-Ciocalteu method (Singleton & Rossi, 1965), based on complex formation of molybdenum-tungsten bleu. The samples were allowed to react with Follin-Ciocalteu'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.

Sample Analysis

Total phenolic content were measured using the modified Folin-Ciocalteu method (Andrew, 2012). About 0.01g samples were diluted with distilled water to gain 0.01g/ml of the sample concentration. Then 0.4ml (400µl) was pipette from stock solution into a test tube. After that, 2ml of 50% Folin-ciocalteu's Reagent were added into the mixture and vortex for 5 minutes. Next, 4ml of 5% Na₂CO₃ were added and placed the test tube in the dark for 1 hour. After 1 hour, the mixture were measured the absorbance at 765 nm with a UV-Visible spectrophotometer (Shimadzu, UV-18800) with distilled water as a blank. The total phenolic content was calculated using gallic acid (GA) calibration curve (50-500 mg/ml). Data were expressed as gallic acid equivalents (GA)/g of extracts averaged from 3 measurements. The TPC of samples were calculated based on the equation below:

$$y = mx + c$$

Where, y = absorbance at 765nm; x = total phenolic content

2.6 Determination of Antioxidant DPPH assays

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 (A. Khalaf et al,2008). The dilute working solutions of the test extracts were prepared in methanolic solution to give the concentration 1mg/ml.

Preparation of DPPH solution

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

DPPH scavenging activity

DPPH concentration is reduced by the existence of an antioxidant at 517nm and the absorption gradually

disappears with time. The spectrophotometer was used to determine the antioxidant activity. From the dilution of 0.02 ml of sample was added with 0.8ml of methanol and 2ml of DPPH solution. The mixture was shaken using vortex for 30 seconds and leave for 30 minutes in dark condition (Molyneux et al, 2004). The reduction and IC₅₀ were calculated. The free radical scavenging activity (% inhibition) was calculated using the formula given below:

$$\% \text{ inhibition} = \frac{(\text{absorbance blank} - \text{absorbance sample}) \times 100}{\text{Absorbance blank}}$$

Extract concentration providing 50% inhibition (IC₅₀) calculate from the graph plotted of inhibition percentage against extract concentrations.

3. Results and Discussions

Yield of samples.

The yield of *E. ridleyi* and *C. asiatica* extraction were 25.14% and 18.37% (Table 1) respectively.

Table 1: The percentage of yield extraction of *Euodia ridleyi* and *C. asiatica*

Samples	Percentage of crude extract (%)
<i>E. ridleyi</i>	25.14 ± 0.07
<i>C. asiatica</i>	18.37 ± 0.19

Phytochemical screening of plant materials

Different phytochemicals test were used to identify the phytochemical present in the samples. According to the result as in Table 2, the screening for phytochemical of the plants studied showed the presence of all phytochemical.

Table 2: Phytochemical Screening of *Euodia ridleyi* and *C. asiatica*

Samples	Flavonoids	Taninns	Saponins	Terpenoids	Phenolic compound
<i>Euodia ridleyi</i>	++	++	+	+++	+++
<i>C. asiatica</i>	++	+++	++	+++	+++

'+++' sign indicates the compounds strongly present, '++' sign indicates moderately present, '+' sign indicates weak present and '-' sign indicates the compounds are absent.

Total Phenolic Contents

Table 3 showed the result for total phenolic contents found in both plant leaves extraction. It is found that the total phenolic content for *E. ridleyi*, calculated from the calibration curve (R² = 0.99932) was 44.56 ± 0.35mg GAE/g, higher than *C. asiatica* extracts with 20.97 ± 0.53 mg GAE/g. The summarized of the results for the extraction of total phenol contents are as below.

Table 3: Total phenolic contents (mgGAE/g) value of *E. ridleyi* and *C. asiatica*

Extract	Phenol contents (mgGAE/g)
<i>E. ridleyi</i>	44.56 ± 0.35
<i>C. asiatica</i>	20.97 ± 0.53

Total Flavonoid Contents

The result for total flavonoid contents of aqueous extraction are shown in Table 4. According to the table, the total flavonoid contents calculated from the calibration curve (R² = 0.97169) obtained in *C. asiatica* and *E. ridleyi* extracts

was 1.51±0.09 mgQE/g and 0.14±0.03 mgQE/g respectively.

Table 4: Total Flavonoid contents (mgQE/g) value of *E. ridleyi* and *C. asiatica*

Extract	Flavonoid contents (mgQE/g)
<i>E. ridleyi</i>	0.14±0.03
<i>C. asiatica</i>	1.51±0.09

Radical scavenging activity assay (DPPH)

The DPPH analysis is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical (Blois et al., 1958; Singh et al., 2008). DPPH gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, then absorption reduces and the DPPH solution is decolourised from deep violet to light yellow, which is monitored spectrophotometrically. The degree of reduction in absorbance measurement is expressing the radical scavenging (antioxidant) capability of the extract.

The results for aqueous leaves extract obtains in both sample are shown in Table 5. *E. ridleyi* contains 5.04 ± 2.17 mg/ml and *C. asiatica* was 5.85 ± 0.17 mg/ml, thus, the scavenging activity capacities for both sample were less than BHT (0.19 ± 0.01 mg/ml).

Table 5: IC₅₀ (mg/ml) value of *E. ridleyi*, *C. asiatica* and BHT

Extract	DPPH IC ₅₀ (mg/ml)
<i>E. ridleyi</i>	5.04 ± 2.17 ^b
<i>C. asiatica</i>	5.85 ± 0.17 ^b
BHT	0.19 ± 0.01 ^a

4. Conclusion

On basis of the results obtained in the present studies, it was concluded that *E. ridleyi* and *C. asiatica* extract contains of flavonoid, tannin, phenol content, terpenoid and saponin. In addition, the TPC of *E. ridleyi* (44.56 ± 0.35) was higher than *C. asiatica* (20.97 ± 0.53) but *C. asiatica* (1.51±0.09) contains higher in TFC than *E. ridleyi* (0.14±0.03). Not all phytochemical are responsible for the antioxidant activity. In other words that even if the plant tested positive for most phytochemical, it could not show the highest antioxidant activity. Further studies are needed for the isolation and identification of bioactive compounds responsible for antioxidant activity.

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