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Evaluation of the DPPH Radical Scavenging Activity, Total Phenolic Content and Total Flavanoid Content of Different Solvent Extracts of *Catunaregam tomentosa* (Blume ex DC) Tirveng Leaves

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ABSTRACT

Catunaregam tomentosa (Blume ex DC) Tirveng is commonly known as Khet in Thailand and Bisa Ular or Badang in Malaysia. The tree is widely distributed in the north-east region of Thailand while in Malaysia the tree usually grows in the open waterfront area at Terengganu. This plant belongs to the Rubiaceae family, and the genus *catunaregam* has interesting pharmacological activities such as anti-inflammatory, antispasmodic, antidysenteric, antifertility and immunomodulatory. In this study, the leaves were extracted using dichloromethane, ethyl acetate and ethanol. Total phenolic was determined by Folin-Ciocalteau method are 115.0 ± 1.135, 340.8 ± 3.209 and 30.24 ± 1.702 mg GAE/g with dichloromethane, ethanol, and ethyl acetate, respectively. Total flavonoid was determined by the aluminium chloride calorimetric method are 47.01 ± 0.577, 56.78 ± 1.970 and 40.89 ± 1.079 mg QE/g with dichloromethane, ethanol, and ethyl acetate, respectively. Meanwhile, its antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The ethanolic extract was found to have the highest percentages of phenolic and flavonoid content. The ethanolic extract also demonstrated strong DPPH scavenging activity with IC50 at 20.07 ± 0.51µg/mL.

Keywords: *Catunaregam tomentosa*, phenolics, flavonoids, antioxidant

INTRODUCTION

Antioxidant activity is one of the most reported bioactive properties in medicinal plants. The plant that has an antioxidant agent contains catechin, eugenol, cinnamaldehyde, carvacrol, gallic acid, *p*-coumaric acid and ellagic acid, can inhibit the oxidation reaction process and scavenge the free radicals (Chan et al., 2016) The free radicals have been implicated in the development number of disorders including cancer, cardiovascular diseases, diabetes, neurodegeneration, and inflammation (Chan et al., 2016). Since the medicinal herbs are one of the

sources that are rich in phenolic compounds, therefore they have recently been investigated for their effectiveness and efficiency as an antioxidant agent (Dahham et al., 2015; Iqbal et al., 2015)

Catunaregam tomentosa (Blume ex DC) Tirveng is commonly known in Thailand as Khet, or in Malaysia as 'Pokok Bisa Ular' or 'Badang'. The plant is found throughout in the north-eastern region of Thailand while it typically grows in Malaysia on the bris soil. It belongs to the Rubiaceae family, and the genus *Catunaregam* includes 12 species distributed across Southeast Asia and Africa's tropical and subtropical regions (Kaennakam et al., 2018). This genus has important pharmacological roles such as antibacterial, infectious, antioxidant, antispasmodic, antidysenteric, antifertility, immunomodulatory, nephrotoxicity properties, antifeedant, antifungal, analgesic and antileukemia. They are been used to treat diabetes mellitus, cancer and tuberculosis (Adikay & Sravanthi, 2016; Kaennakam et al., 2018; Li et al., 2015; Thimabut et al., 2018; Weerapreeyakul et al., 2016). Previous phytochemical investigation on this genus revealed the presence of triterpene saponins, iridoid glucosides, coumarin glucosides, lignans, phenolics compounds, glycerols, carbohydrates, tannins, fats, fatty acids, oils, phytosterols, glycoside and nomeolignans (Gao et al., 2011; Martins & Nunez, 2015; Varadharajan et al., 2014). Although the genus of *Catuneragam* has been well established on their potential biological activities, there is still lack of research focusing on the potential pharmacological properties of *C. tomentosa* and the secondary molecules concerned from this plant that contribute to the activities. Therefore, the objectives of this study to evaluate the antioxidant properties of *C. tomentosa* leaves using different extraction solvents through DPPH radical scavenging activity assay and to determine the total phenolic content and total flavonoid content of the extracts.

MATERIALS AND METHODS

Collection of plant material

The leaves sample of *C. tomentosa* was collected from Taman Ilmu in Kampung Tembila, Besut Terengganu. The random sampling technique was applied, which was young, and old leaves were mixed. The leaves were washed to remove the impurities before being oven-dried at the temperature 40ºC for 24 hours. The sample was ground into the powder. It was kept in a glass vial and was stored at the -80ºC freezer until further used.

Leaves extract preparation

The powdered material was macerated in the 100% dichloromethane, ethanol, and ethyl acetate with ratio 1:50 sample to solvent ratio at room temperature (22-24ºC) for 24 hours. The mixture was sonicated for 10 minutes at the temperature 37ºC. The mixture was centrifuged at 5500 rpm for 5 minutes before filtered through Whatman Filter No.1. The extraction procedure was repeated three times, and the filtrates were pooled together before was evaporated to dryness using rotatory evaporator (Heidoph, Germany). The extract was stored in -20ºC freezer before further use.

Total phenolic content

The total phenolic content was determined by Folin-Ciocalteau method described by Zhang et al., (2006) with slight modifications. A different concentration of gallic acid (0.0625-1.0000 mg/mL) in methanol was prepared and been used as standard. 25 µL of different concentration of standards were mixed with 725 µL of distilled water before being mixed with 125 µL of Folin-Ciocalteau reagent. After that, 500 µL of 20% sodium carbonate was added into the mixture. The mixture of different concentrations of the standard was vortexed and allowed to stand 85 minutes at room temperature. 100 µL of the mixture were transferred to the 96 well plate. The absorbance was read at 765 nm using a microplate reader (Multiskan Go,ThermoFisher). The steps were repeated for the sample, and the total phenolic content (TPC) was expressed as the gallic acid equivalent (GAE) in milligram per gram of dry weight (mg GAE/g DW).

Total flavanoid content

The total flavanoid content was determined by the calorimetric method of Sharma et al., (2015) with some modifications. A different concentration of quercetin (0.0625-1.0000 mg/mL) in ethanol was prepared and used as standard. 125 μL of different concentration of standards were mixed with 375 μL of 75% of ethanol before being mixed with 25 μL of 10% aluminium chloride. After that, 25 μL of 0.1M of potassium acetate and 700 μL of distilled water were added into the mixture. The mixture of different concentrations of the standard was vortexed and allowed to stand for 30 minutes at room temperature. 100 μL of the mixture were transferred to the 96 well plate. The absorbance was read at 415 nm using a micro plate reader (Multiskan GoThermo Fisher). The steps were repeated for the sample, and the total flavanoid content (TFC) was expressed as the quercetin equivalent (Q) in milligram per gram of dry weight (mg Q/g DW).

DPPH free radical scavenging activity

The DPPH radical scavenging assay was conducted according to the method described by Mahmood et al., (2017) with slight modifications. The assay was performed in 96 well microplates. The sample extract of different concentrations (0-250 μg/mL) was prepared using methanol by dilution. The DPPH solution was prepared by dissolved 3.8 mg of DPPH in 95 mL of methanol. 100% methanol was used as control and quercetin was used as an antioxidant standard. The mixture was left to stand in the darkroom for 30 minutes. The analysis was performed in triplicates. The absorbance of the reaction mixture was measured at 517 nm using a micro plate reader (Multiskan Go, Thermo Fisher). DPPH radical scavenging activities of the sample extracts was expressed as IC50 value. The percentage of inhibition was calculated using this formula.

$$\text{Percentage inhibition of DPPH activity} = (A-B)/A \times 100$$

A = Absorbance of reagent blank

B = Absorbance of test samples

RESULTS AND DISCUSSION

Total phenolic compounds and total flavanoid content of *C. tomentosa*

The presence of phenolic- and flavonoid-rich natural compounds in plants had been identified to contribute to their antioxidant values (Parthasarathi & Park, 2015). In this study, both the total phenolic and flavonoids contents in *C. tomentosa* extracts were evaluated using the Folin-Ciocalteu method and aluminium chloride colorimetric method, respectively. Gallic acid (GAE) was used as the standard for the determination of total phenolic content. In contrast, quercetin (QE) was used for developing the standard curve for total flavonoid content. The ethanolic extract of *C. tomentosa* showed the highest total phenolic and flavonoid content, 340.8 ± 3.209 mg GAE/g and 56.78 ± 1.970 mg QE/g, respectively (Table 1).

Meanwhile, total phenolic and flavonoid content of *C. tomentosa* extract in dichloromethane, and ethyl acetate was at 115.0 ± 1.135 mg GAE/g, 47.01 ± 0.577 mg QE/g, and 30.24 ± 1.702 mg GAE/g, 40.89 ± 1.079 mg QE/g, as determined from the respective assays (Table 1). Phenolics compounds possess plenty of biological activity such as antioxidant, antimutagenic, and anticarcinogenic (Marinova et al., 2005). There is an abundance naturally occurring plant phenolic, and about half of are flavonoids which mainly contribute to antioxidant effects. The *C. tomentosa* leaves extracts exhibit potential components, as shown in this analysis suggested to contribute to antioxidant activity.

Table 1. Total phenolics and flavanoids of *C.tomentosa* leaves extracts

Extracts	Total Phenolic content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
Dichloromethane	115.0 ± 1.135	47.01 ± 0.577
Ethanol	340.8 ± 3.209	56.78 ± 1.970
Ethyl Acetate	30.24 ± 1.702	40.89 ± 1.079

DPPH free radical scavenging activity of *C.tomentosa*

The antioxidant activity of *C. tomentosa* leaves extract was also evaluated using a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). This method measured antioxidant capability by determining the decrease in absorbance reading that was caused by the colour change of the DPPH radical from purple to yellow upon reduction by an antioxidant or radical species. The IC50 values, which correlated to the concentrations of the sample that caused 50% of DPPH disappearance, were determined graphically from the plot of absorbance reading against sample concentration. The result showed that the ethanolic extract of *C. tomentosa* caused 50% inhibition of DPPH radical scavenging activity at the concentration of 20.07 ± 0.51 μg/mL. Meanwhile, 50% inhibition activity in dichloromethane and ethyl acetate extract of *C. tomentosa* was at 82.33 ± 0.58 μg/mL and 159.25 ± 5.38 μg/mL, respectively (Table 2).

Table 2. Antioxidant activity of *C.tomentosa* leaves extracts

Extracts	IC_{50} (μg/mL)
Dichloromethane	82.33 ± 0.58
Ethanol	20.07 ± 0.51
Ethyl Acetate	159.25 ± 5.38
Quercetin	7.33 ± 0.85

In comparison to the positive controls, the antioxidant activity of *C. tomentosa* was about 7.33 ± 0.85 μg/mL (Fig. 1). Interestingly, the ethanolic extracts showed stronger inhibition of DPPH molecule as compared to the other extract. The DPPH assay is a simple, acceptable, and most widely used technique to evaluate the radical scavenging potency of plant extracts. Antioxidant positively correlated with phenolic content (Oki et al., 2002) and this suggestion was verified by Heim et al. (2002), Heim et al. (2002) and Rufino et al. (2010) that the amount of total phenolic compounds correlated significantly with the antioxidant capacity. Besides, phenolic compounds could act as antioxidants agents due to the phenolics hydroxyl groups acts as hydrogen-donating antioxidants and hydrophobic benzoic rings to inhibit some enzymes such as various cytochrome P_{450} isoforms, lipoxygenases, cyclooxygenase, and xanthine oxidase involved in radical generations (Fukumoto & Mazza, 2000; Parr & Bolwell, 2000) As previously shown where high phenolic content in ethanolic extract of *C. tomentosa* resulted in strong antioxidant activity suggested correlating between phenolic and antioxidant effects of *C. tomentosa*.

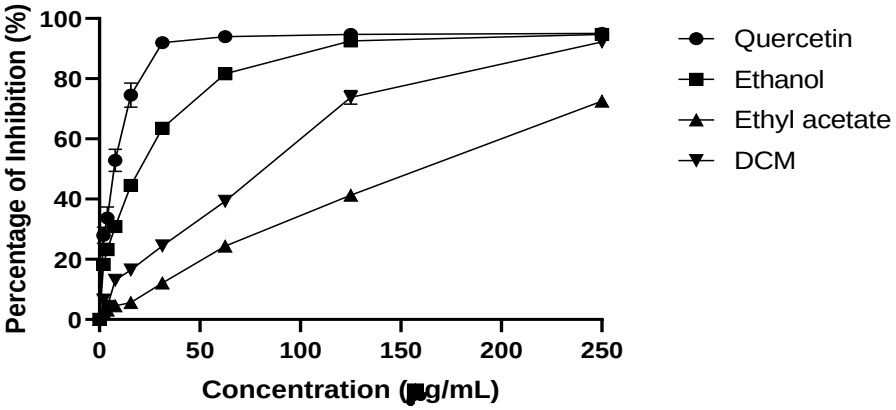

Fig. 1. Antioxidant activity of *C. tomentosa* leaves extracts in different solvents type and quercetin. Ethanolic extract showed the strongest antioxidant activity at IC50 concentration 20.07 ± 0.51 µg/mL and IC50 of quercetin at 7.33 ± 0.85 µg/mL. Each point represents the mean of the results of three independent experiments. Error bars represent means ± S.E.M. of the three independent experiments.

CONCLUSION

The phenolic and flavonoid content of *C. tomentosa* leaves extract revealed the potential of this plant regardless of the solvent used. However, this study disclosed that ethanolic extract has the highest phenolics and flavonoids content and resulted in strong DPPH radical scavenging activity. Further chemical profiling suggested being executed in future.

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