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Total phenolic content (TPC) in *Catharanthus roseus* and *Clitoria ternatea* leaves extract

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Abstract

Catharanthus roseus or "Kemunting Cina" and *Clitoria ternatea* or "Bunga Telang" had a long reputation as a traditional remedy in Southeast Asia. One of the important components that contribute to these plants' remedial capabilities is the secondary metabolic known as phenolic. In this study, the Total Phenolic Contents or TPC in *C. roseus* and *C. ternatea* leaves extract have been obtained and compared. Leaves from both species were dried and the extract was acquired using 90% ethanol via a rotary evaporator. Two major tests were conducted which are the qualitative test and quantitative test. The qualitative test was done to ensure the existence of phenolic in the samples. Meanwhile, the quantitative test is to measure the concentration of phenolic in the samples. Both samples show a positive result in a qualitative test where the extract of the sample changed from green to dark green in the Ferric Chloride test. Next, Folin-Ciocalteu assay acted as the quantitative test to determine the TPC in the samples, and the absorbance was measured at 760 nm. The test was triplicated to ensure the consistency of the results. The total phenolic content for *C. roseus* is 36.33 ± 0.935 mg/GAE and *C. ternatea* is 7.36 ± 0.046 mg/GAE. The TPC in *C. roseus* is higher than *C. ternatea*. The t-test shows that there is a significant difference at $p < 0.05$ in concentration of total phenolic content between both extracts.

1. INTRODUCTION

Catharanthus roseus or *C.roseus* come from the family Apocynaceae and is commonly known as Periwinkle, Old Maid, and "Kemunting Cina" in Malay (Figure 1). This plant is usually used as an ornament plant due to varieties of colours from its flower and its durability toward heat. *C. roseus* is important medicinal plant and the previous researcher found that the plant extract can synthesize two terpene Indole alkaloids that have the potential to cure cancer (Mishra and Verma, 2017). Based on previous studies, researchers who study the medicinal properties of *C. roseus* discovered that it contains around 400 types of alkaloids present in the plant that can use as agrochemical, fragrance, flavor, food additives, and pesticide (Das and Sharangi, 2017). Although alkaloid is extremely toxic, this substance has big potential in treating cancer. Each part of *C. roseus* has a different type of alkaloids such as in roots and basal stems, such as ajmalicine, Vincente, vincamine, raubasin, reserpine, catharanthine and etc. Meanwhile, *Clitoria ternatea* come from the family name Fabacae (leguminosae) and this plant has many common names such as Butterfly Pea, Blue Pea, Blue Vine, Pigeon Wings, and Mussel Shell Creeper and in Malay "Bunga Telang" (Figure 2). The plant is herbaceous, slender, and tall with climbing vines. It flowers ranging from the color blue to white with yellow at its center and is commonly used for food coloring. According to John et al. (2016), this plant-rich source with pharmacologically active secondary metabolites such as polyphenolic flavonoids, anthocyanin glycosides, pentacyclic triterpenoids, and phytosterols. This plant is resistant to drought and will thrive in years with only 400 mm of rainfall and a dry season of 5-6 months or longer, even if it is heavily weeded (Sarwar et al, 2014). The plant is also known as a medicinal plant and all its plant part (roots, seed, and leaves) can be used as a treatment for inflammation and laxative, treatment for respiratory diseases such as asthma and bronchitis (Manjula et al., 2013). In the modern-day, cancer is one of the diseases on top of the list that can

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cause fatality in humans all over the world. Cancer can be caused by several factors such as environment, social, cultural, lifestyle, hormonal and genetic. In Malaysia, the Chinese ethnic had the highest breast cancer with an ASR of 41.5 per 100,000 women followed by the Indian 37.1, and lastly, Malay with 27.2 per 100,00 women from 2007 to 2011 (Kirubakaran et al., 2017). There are many ways to cure cancer such as surgery, and radiotherapy but the most widely used is chemotherapy. Chemotherapy is effective against most cancer. In the beginning, it was thought that chemotherapy only kills cancer cells. However, there are many side effects of chemotherapy such as fatigue, nausea, and hair loss reported in the patient undergoing this treatment (Kerr et al, 2019). Chemotherapy also kills or damage normal cell during its process. Chemotherapy treatment cost is very high, and it depends on the type of cancer the patient has and the cycle of treatment needed. According to Cancer Cure Malaysia, the cost range for chemotherapy treatment is as low as RM 18,000 to the highest RM350, 500. There is a previous study that state that the old folk uses traditional remedy to cure cancer such as using *C. roseus* (Das and Sharangi, 2017) and *C. ternatae* (Gollen *et al*., 2018). *C. roseus* and *C. ternatea* can be used as an alternative way to treat or reduce cancer growth and metastasis because the plants may contain the phenolic antioxidant. Antioxidants are unique compounds that protect cells of humans, animals, and plants from the harmful effects of free radicals such as reactive oxygen species, ROS. An imbalance between antioxidants and free radicals causes oxidative stress and can cause cell damage (Ali and Neda, 2011). By using the natural approach such as *C. roseus and C. ternatea*, the finding has the potential to help cancer patients economically as natural approaches such as herbal treatment can easily obtain the plant growth wildly everywhere and also can be purchased at nurseries at a reasonable price. Herb can decompose in at short time, so it has a lower cost to handle waste compared to the commercialized drug.

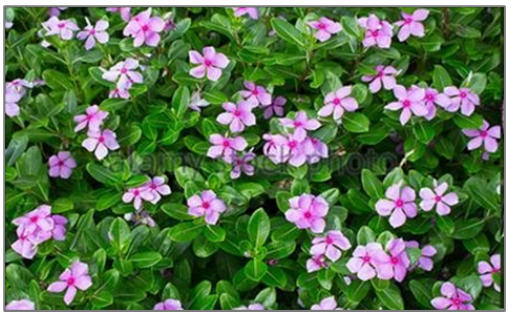

Figure 1: *Catharanthus roseus*'s flower and leaves. (Adopted from Taher *et al*, 2017)

Figure 2: *Clitoria ternatea*'s flower and leaves. (Adopted from Jamil *et al*, 2018)

2. MATERIALS AND METHODS

2kg plant sample leaves had been purchased from the nursery and collected from the garden. The two plant species have been separated between two boxes. Then, the plant leaves were cleaned with tap water to remove any soil (Kaur, 2014) and cleaned again using distilled water. After that, the leaves were placed into the universal oven to dry the leaves for 48 hours at 60 ºC. After 48 hours, the leaves were ground and sieved to get a uniform fine powder. The fine powder from each plant was placed inside two different Schott bottles. Each bottle had been labeled with the species' name. Next step, the ground leaves were soaked with ethanol at a ratio of 1:10 with 10 g dried leaves powder with 100 ml ethanol with 90% concentration (Ramya, 2008) at room temperature for 24 hours. After the sample was soaked for 24 hours, the solutions were filtered by using Whatman filter paper no 1 to obtain the filtrate and the leaves that have been filtered are discarded (Shalini and Sampathkumar, 2012). Then, each filtrate was evaporated by using a rotary evaporator at 40 ºC for one hour until crude extract was acquired. For the qualitative screening, the ferric chloride test is done to detect the presence of the phenolic compound in the plant extract. According to the method by Kumar (2015), using a Pasteur pipette, a small amount of extract was obtained, and the extract was dissolved in 5 ml of distilled water. Then, a few drops of 5% ferric chloride solution were added to a mixture of distilled water and leaf extract. The solution was left for 10s to let the reaction happen and if there is a presence of the phenolic compound, the colour of the solution will change from violet to blue, green, or red depending on the nature of the phenolic compound. Meanwhile, for quantitative screening, a standard curve is necessary to calculate the amount of phenolic content absorbance. The standard curve was prepared by using different concentrations of the standard (Pallab, *et al*., 2013). The standard used in

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this research is Gallic acid. The positive control used in this research is butylated hydroxytoluene, BHT. The negative control for the research is ethanol. The step used by Nisar *et al*. (2017), with slight modification where 100mg of gallic acid was dissolved in 100 ml ethanol and then diluted to 20, 40, 60, 80, and 100 mg/ml was used. The curve was obtained by measuring the absorbance of gallic acid at 765nm with a UV spectrophotometer. The total phenolic content of the leaf extract was measured using the Folin-Ciocalteu assay. Shahin et al. (2016) method was used as a reference for the assay. 40 µl of the extract was added with 200 µl diluted Folin's reagent (1:10) and shaken properly. After 8 min, 600 µl of 7.5% sodium carbonate was added to the mixture to create the basic condition for the redox reaction between the phenolic compound and Folin's reagent to occur. Then, the mixture was diluted to 4 ml with distilled water. After that, the mixture requires 60 minutes of incubation. After 60 minutes of incubation, the absorbance sample was measured using a UV-VIS spectrophotometer at 765 nm and the results were expressed as mg /GAE. The experiment results were expressed as mean ± standard deviation of three triplications of each sample. The data were analyzed in T-test distribution by using the IBM SPSS program (22nd edition) to compare the total phenolic content of the leaves between *C. roseus* and *C. ternatea*.

3. RESULT AND DISCUSSION

Table 1 shows both leaves extract of samples contained phenolics in qualitative screening test. These results are in line with other studies that have performed qualitative phytochemical screening on the ethanolic extract of both samples. This can be seen by the finding made for ethanolic sample extract of *Catharanthus roseus* by Kabesh et al. (2015) and *Clitoria ternatea* by Kumari (2013). Solution ferric chloride gives several organic derivatives to colours. Phenolic compounds are the most widely distributed secondary metabolites in the plant kingdom, although the variety of compounds present differs according to the phylum being considered (Lattanzio, 2013). For phenolics, when its react to ferric chloride, phenoxide ion, ArO-, from phenolic compound forms coordinated bonds with ferric ion, Fe, ferric chloride, resulting in a colour change. When yellow-coloured ferric chloride solution is added to extracts containing phenolics, the color of the solution will change from violet to blue, green, or red depending on the nature of phenolic compound. The colour of the extract for both leaves extract was green. After each extract was diluted with 5 ml of distilled water, the colour of the leaves extract became lighter. The green-colored of both extracts changed to light green. After that, when yellow coloured of 5% ferric chloride was added into diluted extract the colour changed immediately to dark green. Table 2, shown the colour changes to dark green shows that the presence of phenolics in leaf extract of *Catharanthus roseus* and *Clitoria ternatea*.

Table 1: Result of qualitative screening for samples including the positive and negative control.

Plants/ Sample	Phenolics Qualitative Screening
Catharanthus roseus	+
Clitoria ternatea	+
BHT (Positive control)	+
Ethanol (Negative control)	-

Table 2: Colour of sample changes into the dark green for ferric chloride test.

Catharanthus roseus	*Clitoria ternatea*
	

3.1 Statistical analysis

For the quantitative screening, total phenolics content was determined with the Folin- Ciocalteu assay. Gallic acid has been used as a standard for the calibration curve. The total phenolics content were expressed as mg GAE/g using the standard curve equation: $y = 0.0124x + 0.521$, $r2 = 0.9901$, where y is absorbance at $\lambda = 765$ nm and x is total phenolics content in the extract.

Meanwhile Table 3 shows the total phenolics of leaves of *C. roseus* and *C. ternatea* that were measured by Folin's Ciocalteu assay, in terms of gallic acid equivalent (GAE). The total phenolic content in the *C. roseus* is 36.33600 ± 0.935313 mg/GAE and *C. ternatea* is 7.35767 ± 0.046188 mg/GAE. *C. roseus* yielded higher TPC than *C. ternatea* and each of it is significantly different from the other, $p<0.05$.

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Table 3: Total phenolic or TPC for samples.

Plant Species	Total Phenolic (mg/ GAE)
Catharanthus roseus	36.33 ± 0.935
Clitoria ternatea	7.35 ± 0.046

In the study of Pham et al. (2016) showed total phenolic content on *C. roseus* was 19.21 ± 1.15 mg / GAE. The result from the study was different from this study might be due to different thermal drying methods. Pham used the hot air-drying method at 80 ºC while this study used oven drying at 60 ºC. The bioactive components and antioxidant properties of both samples are significantly affected by drying methods. The heat can decrease the number of phytochemicals due to stress applied to the plant tissue causing loss of water, hence it will cause the degradation of plant tissue (Abu-Ghannam et al, 2011). The analysis was done by Lakshmi et al. (2014) yielded a total of 58.5 mg GAE in the extract of *C. ternatea* leaves. However, Lakshmi et al. (2014) used 90% methanol as a solvent for extraction, but in this study, the leaves of *C. ternatea* were extracted with 90% ethanol. There is a difference in the yield of total phenolic content because different type of solvent has a different polarity that suits various plant species. According to Naczk and Shahidi (2006), solvent polarity is necessary to recover phenolics from plant material by enhancing phenolic solubility during solvent extraction processes. Methanol has a higher result because, in more polar solvents, phenolic is often extracted in greater quantities. It is important to choose the solvent that has high solubility to compound to maximize the amount of phytochemicals that can be extracted. The extraction method plays a role to ensure the quantity of the extraction. This is because regardless of plant sample varieties, the usage of optimal solvent and heat in extraction is vital. This was shown by Sulaiman et al. (2011) where the samples were extracted by using distilled water while in this study, 90% ethanol was used. The samples were boiled first before being filtered while in this study, the samples received heat treatment only up to 60ºC. Consequently, thermal treatment to plant samples will instigate biological, physical, and chemical modification that leads polyphenols compounds to release from their bond, degraded, and oxidize (Palermo et al., 2014). Moreover, boiling as heat treatment to the plant samples is claimed to be the least effective method to retain phytochemicals (Minatel et al.,2017). Independent-t-test shows that there are significant differences in total phenolic content between *C. roseus* and *C. ternatea* leaves extract at $p<0.05$. Based on the previous studies there are limited experiments or research that compared phenolic content between both plant species. However, the phenolic varies significantly because the production of secondary metabolites such as phenolic depends on plants. Each plant has different growth processes and specific requirements (Azmir et al., 2013). For example, plants growing in harsh environments produce more antioxidants (Azmir et al. 20130. There is a significant difference in total phenolic content $p<0.05$, where *C. roseus* have higher phenolic content compared to *Clitoria ternatea*. This is because phenolic provides resistance to pesticides and pests, and control processes of germination, growth, and reproduction (Viera da Silva et al, 2016). *C. ternatea* does not require high phenolic because *C. Ternatea* is resistant to drought and will thrive in years with only 400 mm of rainfall and a dry season of 5-6 months or longer, even if it is heavily weeded (Sarwar et al, 2014). This is also maybe because of the help from other secondary metabolites that exist in the plant such as alkaloids.

4. CONCLUSION

In conclusion, the total phenolic in leaves extract of *C. roseus* and *C. ternatea* were successfully obtained and quantified. The data acquired in this study showed that there was a significant different in total flavonoid and phenolic content at $p<0.05$ when it is compared *C. roseus* and *C. ternatea*. It is recommended for further research on finding the suitable solvent and optimum concentration for extraction of *C. roseus* and *C. ternatea*. This is because each plant has its optimal type of solvent and concentration. This is important to increase the maximum yield of phenolic from the extract. Other than that, phenolics have antioxidant properties. Therefore, further research on the effect of the phenolic in *C. roseus* and *C. ternatea* against various types of disease such as cancer can be done. Lastly, it is also recommended to make further research on secondary metabolites such as alkaloids which can be found in leaves. This research is important because other secondary metabolites that can be found have several benefits.

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