Effect of water stress on antibacterial activity, Total Phenolic Content and Total Flavonoid Content of *Clitoria ternatea*

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Abstract. The present investigation was aimed in determining the antibacterial activity of *Clitoria ternatea* that experiencing water stress treatment which comprises of positive control (watered normally for every 24 hours), negative control (no presence of water) and a treatment where *C. ternatea* receive water for every 48 hours. Agar disk diffusion assay had been utilized in order to investigate the measurement of zone of inhibition based on the methanolic leaf extract of *C. ternatea* for each treatment. Plus, calculation had been done for total phenolic content and total flavonoid content, to correlate between the length of inhibition zone. The results show that there is no presence of inhibition zone for all treatments which might be due to the low potency of the methanolic leaf extract, 0.02g/ml. *C. ternatea* that undergo every 48 hours of water presence hold the highest amount of total phenolic content whilst *C. ternatea* that encountered negative control of treatment possess the high amount of total flavonoid content. High quantity of phenolic and flavonoid content can be related with high amount of antioxidant capacity.

1. Introduction

Nowadays, climate change is one of the major problems that could cause a natural disaster. This dreadful event happened due to the elongation period of enhanced greenhouse gases which likely would cause disturbance towards the living organisms on Earth. The main predicament from this event is that drought period will starts to emerge that would eventually give rise to forest fire and global warming occurrence and detrimental effect such as water stress condition towards biodiversity will be emanated [1]. To be clear, drought is known as the unbalanced quantity of water between the production of the nature and the needs of human and environment [2]. Mobile creature would be easy for them to fit in with this situation rather than the immobile one such as plants community. However, there are certain type of plants that can survive this water shortage situation such as *Carthamus tinctorius* and *Cuminum cyminum L.* where the increment of secondary metabolites had occur to make them survived [3, 4]. Thus, a review done by [5] was conducted as *Clitoria ternatea* has the potential as medicinal plants that possess a moderate resistance to water stress level.

*Clitoria ternatea* is known as butterfly pea and had possessed different kind of name throughout the continent such as *Mazerion Hidi, Baslat el-Zuhoor* in Arabic, *Aparajita* in Bengali and Hindi, *die douin Chinese honte* in French, *blaue Klitorie* in German, *clitória-azul, clítoria* in Portuguese, *Koyal* in Punjabi, *Girikarnika* and *Vishnukranta* in Sanksrit, *conchitas papito, azulejo, zapatico de la reina,*
zapotilloin Spanish himmelsärt in Swedish, Kakkanam in Tamil and Dintena in Telugu. This medicinal plant has synonyms such as Clitoria albiflora Mattei, Clitoria bracteata Poir., Clitoria mearnsii De Wild., Clitoria tanganicensis Micheli, Clitoria zanzibarensis Vatke [6]. Clitoria ternatea Linn. belongs to the family Fabaceae and is a type of persistent perennial leguminous twinning herbal medicinal plant. C. ternatea mostly being utilized as pasturage, hay-making, companion crop and ornamental [7, 8] but however, butterfly pea had been used for traditional practices of Indian Ayurvedic, Siddha, Unani [9]. Furthermore, some previous paper had emphasized that C. ternatea was originated from tropical Asia and some said it was first emerged in the Caribbean. Nevertheless, its genuine origin remains unclear due to the ample cultivation and naturalization around the Earth.

Clitoria ternatea possess a trait of climbing or trailing which grow from a woody rootstock. Based on Figure 1 and 2 below, it leaves imparipinnate with 2-4 pairs of leaflets and a terminal leaflet with ovate to elliptic-oblong type of shape. C. ternatea are mostly hairless above and pubescent below. Flowers axillary and sometimes can be solitary or 2 together which is resupinate, large and showy and mostly own a bright blue colour. Pod linear oblong, 6-13 cm long, flattened and mucronate at the apex, hairless or finely pubescent [10]. Every parts of C. ternatea consists of valuable and different type of active compounds which can be directly contribute to pharmacological uses such as antioxidant, hypolipidemic, anticancer, anti-inflammatory, analgesic, antipyretic, antidiabetic, CNS, antimicrobial, gastro-intestinal antiparasitic, insecticidal and many more. Such active compounds that embedded inside this medicinal plant are nicotiflorin (flavonoid), tannin, phlobatannin, flavonoid, anthraquinone, alkaloid, cardiac glycosides, volatile oil, steroid, terpenoid, resins, starch, taraxerol, taraxerones, flavonoid, saponins, proteinand carbohydrates [10].

Figure 1. Seeds and pods of C. ternatea
Figure 2. Flowers and twinning stems of C. ternatea

2. Materials and Methods
On early of May, seeds of Clitoria ternatea were sown in a soil that contain organic manure. After the seeds sample had grown and reached five-leaf stage and mature enough to experienced water stress treatment, 15 pots of matured C. ternatea were chose to encounter water stress treatment that have been carried out for 30 days. 15 plant samples were treated under 3 different types of water stress treatment which are the negative control (no presence of water), positive control (watered for every 24 hours) and a treatment where the plant samples were watered for every 48 hours.

Other After 30 days treatment, C. ternatea leaves were harvested and extracted in order to get the leaf crude extract. For extraction process, cold maceration technique was applied since it is applicable in hindering activity of Gram-positive and Gram-negative bacteria where methanol solvent and powdery form of the leaves part were combined. The extraction was filtered by using the standard filter
paper. Soaking and filtration processes were repeated until the colouration of the leaves C. ternatea decolourized. The methanolic crude extracts were vaporized to dryness through the application of the fume hood [11]. The extracts will be kept inside the -20°C freezer. For bacteria growth preparation, both Bacillus cereus and Pseudomonas aeruginosa were cultivated on Mueller-Hinton agar at 37°C by using the streak plate techniques. Furthermore, for antibacterial testing preparation, agar disk-diffusion assay was performed and inoculum need to be prepared first, by following the standard of McFarland techniques. Pour-plate method was utilized by mixing the inoculum with Mueller-Hinton agar under suitable heat for the bacteria suspension to live, 37°C -0°C. Filter paper disc with a size of 6mm were soaked with methanolic leaf extract of C. ternatea and were placed on top of the agar. The agar plates were stored inside the incubator at 37°C and zone of inhibition were recorded.

Next, the determination of total phenolic content and total flavonoid content where gallic acid and rutin compound were used as standardization preparation, respectively. Folin-Ciocalteau reagent were used to determine the total phenolic content of methanolic leaf extract of C. ternatea and to correlate between the antibacterial activity that cause the zone of inhibition. Briefly, 200μL of crude extract (1 mg/mL) were made up to 3mL with distilled water and mixed thoroughly with 0.5mL of Folin–Ciocalteau reagent for 3 min and 2mL of 20% (w/v) sodium carbonate were added. The mixture was allowed to stand for a further 60 min in the dark, and the absorbance was measured at 650nm. The total phenolic content was calculated from the calibration curve. To get the total phenolic content, the formula C = C1 x V/m was applied. As for total flavonoid content, aluminium chloride colorimetric method was utilized where 50μL of crude extract (1 mg/mL ethanol) were mixed with 1mL of methanol. 4mL of distilled water and then 0.3mL of 5% NaNO2 solution were added. After 5 min, 0.3mL of 10% AlCl3 solution were poured and left for 6 min. 2mL of 1 mol/L NaOH solution were added and the final volume of the mixture was brought to 10mL with distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510nm [12]. Data were collected by analyzing the effectiveness of antibacterial activity of leaf extract of C. ternatea under water stress treatment for 30 days with 3 different water shortage conditions. All analyses were performed in triplicate and measured by using mean ± SD.

3. Results and Discussion

Clitoria ternatea was subjected to treatment of water stress for 3 different levels which were every 24 hours (positive control), every 48 hours (treated) and no presence of water (negative control). The responses on its antibacterial activity and bioactivity of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were recorded and discussed.

Furthermore, results on antibacterial activity, based on Table 1, it can be seen that there is no inhibition zone for all treatments of water stress that have been carried out. This may be due to the lack potency of the methanolic leaf extract which is only 0.02g/mL where the dosage is not potent enough to kill bacterial colonies for both type of bacteria species. Plus, there is no specific findings about the influence of Clitoria ternatea leaf extracts against the gastrointestinal system since both type of bacteria species produced toxins that might cause infections at the gastrointestinal area including pneumonia, infections in urinary tract, stomach ache and others related [13].

Based on Table 2, it shows that all sample group B possess the highest amount of total phenolic content whilst sample group C hold the highest amount of total flavonoid content. This can be explained that higher amount of phenolic and flavonoid content indicates higher amount of antioxidant capacities [14]. The greater the rate of water stress treatment, the higher the rate of total phenolic and total flavonoid content. However, due to zero presence of inhibition zone, there might be technical errors regarding the antibacterial activity assay where the methanolic leaf extract of C. ternatea are not potent enough. Findings said there is no specific level of toxicity for C. ternatea as it states that the extract was found safe even at the dose of 2g/kg body weight in rats [15]. The problem is that, the minimum dry weight of C. ternatea leaves attained, that undergo water stress event are only 0.04g. Shortage of the sample makes it quite hard to redo the experiment over and over again. Table 2 depicts an outlier number of total phenolic content and total flavonoid content, respectively which might be
due to the improper pipetting procedures.

**Table 1.** Antibacterial activity of *C. ternatea* based on 3 different water stress treatment. Analyses were accomplished in triplicates.

| Bacteria                      | Samples                | Positive control | Watered for every 48 days | Negative control |
|-------------------------------|------------------------|------------------|---------------------------|-----------------|
| *Bacillus cereus*             |                        |                  |                           |                 |
| *Pseudomonas aurigenosa*      |                        |                  |                           |                 |

Table 2. Total Phenolic Content GAE (mg/g) and Total Flavonoid Content GAE (mg/g) of *Clitoria ternatea* extracts subjected to different levels of water treatment. All samples were expressed as mean ± SD.

| Water stress treatment | Sample | Total Phenolic Content GAE (mg/g) Mean ± SD | Total Flavonoid Content GAE (mg/g) Mean ± SD |
|------------------------|--------|---------------------------------------------|---------------------------------------------|
| Positive control       | A1     | 3.08933 ± 0.48587                           | 7.69896 ± 1.29843                           |
|                        | A2     |                                             |                                             |
|                        | A3     |                                             |                                             |
|                        | A4     |                                             |                                             |
| Watered for every 48 hours | B1 | 3.33146 ± 0.02355                           | 14.4735 ± 0.2725                            |
|                        | B2     |                                             |                                             |
|                        | B3     |                                             |                                             |
|                        | B4     |                                             |                                             |
| Negative control       | C1     | 2.64613 ± 0.16798                           | 13.127 ± 1.10525                            |
|                        | C2     |                                             |                                             |
|                        | C3     |                                             |                                             |
|                        | C4     |                                             |                                             |
4. Conclusion
In conclusion, it can be emphasized that there are no definite results of antibacterial activity which indicates that C. ternatea that undergo water stress treatment are not that good enough to fight bacterial infections at the gastrointestinal system. Furthermore, the higher the stress were given onto the plant, the higher the rate of total phenolic content and total flavonoid content.

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