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A comparison of Bradford, Lowry and UV-280 methods to determine the protein content from green and red *Christia vespertilionis* leaves

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Abstract
Christia vespertilionis comprises two types, namely green and red leaves. Both have biological compounds that promote anti-cancer and anti-inflammatory activities. Protein content has been used as a parameter to analyse the potentials of *C. vespertilionis* as a medicinal plant. Thus, this study is aimed at comparing three spectrophotometric methods (Bradford, Lowry and UV-280) to determine the protein content of *C. vespertilionis* leaves in a precise and accurate way. The protein content in the purified extract of green supernatant (GS), green pellet (GP), red supernatant (RS) and red pellet (RP) of *C. vespertilionis* leaves was determined by Bradford's, Lowry's and UV-280 methods, respectively. GP contained the highest protein content for all the methods with Bradford as the highest sensitivity at 4.51±0.040 mg/ml. Whilst, Lowry in the moderate sensitivity, at 0.528 ± 0.005 mg/ml and UV-280 in the lowest sensitivity with 0.293 ± 0.003 mg/ml protein content. Based on the results, the Bradford method shows more reliable protein values than Lowry and UV-280, due to high sensitivity and precision. This study is of much significance as it helps in acknowledging the most accurate and precise method for determining protein content.

Keywords: Bradford, *C. vespertilionis*, Lowry, protein content, UV-280

Introduction
Christia vespertilionis is referred to as butterfly wings due to the similarity of their leaf structure to that of a butterfly wing. It is hard to survive in extreme weather but easy to adapt to shelter conditions. The leaf's origins from Southeast Asia regions include Malaysia, Indonesia, Thailand, Vietnam, Cambodia and China. This plant has been studied extensively for its biological compounds and its effect on health as an antioxidant, anticancer, antidiabetic, anti-plasmodial and anti-proliferative [1, 2, 3, 4]. It is also used to treat malaria fever, tuberculosis, bronchitis, tonsils, scabies, muscle weakness and poor blood circulation [5, 6, 7]. Determination of protein content using spectrophotometric method is widely and commonly used in many areas related to laboratory work. The spectrophotometric method has its disadvantages in determining protein contents that easily attracts all types of protein and other unrelated molecules rather than a specific one [8]. Thus, it is established from literature that, Bradford 1976 [9] and Lowry et al. 1951 [10] are the easiest, sensitive and quickest methods. Bradford is based on the shift of a dye that attracts the concentration of protein at 595 nm while Lowry is based on the reactivity of peptide bonds at 660 nm. Bradford is believed to be more sensitive to proteins with high arginine, lysine and histamine content. Comparatively, the UV-280 [11] method has a lower sensitivity to proteins and can easily cause an error.
These three methods have their characteristics and limitations. Thus, it is necessary to perform a comparison between these methods on target samples to be analysed, with the aim of determining the method that is approachable, convenient and has more possibilities of achieving accurate results. There is a lack of research that focused on comparing the different methods of spectrophotometric in order to analyse protein content in the sample. Therefore, this study aims to compare the protein content of *C. vespertilionis* leaves (green and red) determined by Bradford, Lowry and UV-280 methods as well as investigate which method is more precise and gives more accurate results.

Materials and Methods
Plant material
Green *C. vespertilionis* (Figure 1) and red *C. vespertilionis* (Figure 2) were collected from Floranika Nursery Sungai Buloh, Selangor (Malaysia), located at the latitude and longitude of 3° 13' 6.7764" N, 101° 34' 18.1704" E. The voucher specimen was certified by Dr. Yong Kien Thai from Plant Taxonomy, Rimba Ilmu, University of Malaya. The voucher specimen of green *C. vespertilionis* (KLU 50026) and red *C. vespertilionis* (KLU 50025) were placed at the herbarium of University of Malaya.

Fig 1: Green *C. vespertilionis*

Fig 2: Red *C. vespertilionis*

Partial purification of protease enzyme

20 g of green *C. vespertilionis* leaves and red *C. vespertilionis* leaves were washed thoroughly with tap water and grinded with 400 ml pre-chilled 0.1 M sodium phosphate buffer at pH 7 separately. The crude extracts were filtered through cheesecloth to remove the suspension. Afterwards, the crude extracts were centrifuged at 9000 rpm at 4 °C for 15 minutes to remove impurities. The supernatant was collected and stored at 4 °C until the next test.

Next, ammonium sulfate precipitation was performed for green *C. vespertilionis* leaves in the saturation of 100 % (34.85 g / 50 ml) and red *C. vespertilionis* leaves in 80 % (25.8 g / 50 ml) based on ammonium sulfate precipitation table as shown in appendix. Ammonium sulfate, $(NH_4)_2SO_4$ was dissolved with crude extracts and stirred until a homogeneous state, for overnight at 4 °C. Afterwards, centrifugation was done at 9000 rpm for 15 minutes at 4 °C. The supernatant and pellet were collected for each saturation of both green and red leaves and then, were re-suspended with 10 ml pre-chilled 0.1 M sodium phosphate buffer at pH 7.

Dialysis was performed for all four samples, which are green leaves supernatant (GS), green leaves pellet (GP), red leaves supernatant (RS) and red leaves pellet (RP) of *C. vespertilionis*. Each sample was pipetted into the dialysis membrane and kept in a beaker with 300 ml of pre-chilled 0.1 M sodium phosphate buffer at pH 7 in a magnetic stirrer plate. The buffer was changed every 2 hours within 4 hours and then kept overnight to ensure ammonium sulfate salt was completely removed. Consequently, the purified samples were centrifuged at 9000 rpm for 15 minutes at 4 °C and the supernatant was collected and kept at 4 °C.

Determination of protein content

The protein content in all samples during the purification of ammonium sulfate precipitation was determined by the Bradford method, Lowry method and UV-280 method. Bradford and Lowry's standard curves were constructed using bovine serum albumin (BSA) at a concentration range of 0.0-1.2 and 0.0-1.0 mg/ml, respectively while UV-280 standard curve was constructed using L-tyrosine at a range of 0.0-0.2 mg/ml. The absorbance was measured using a UV-Vis spectrophotometer (Shimadzu, UV-1700, Kyoto, Japan) at 595, 660 and 280 nm for Bradford, Lowry and UV-280, respectively.

Statistical analysis

All the results were performed using GraphPad Prism 8.0.2, where the two-way ANOVA test was conducted at a significance level of $P < 0.0001$.

Result and Discussion

Figure 1 depicts that protein contents of each method (Bradford, Lowry and UV-280), was performed with calibration curves. The straight lines of Bradford, $y = 0.0762x + 0.0061$ and $R^2 = 0.9698$, Lowry with $y = 0.629x + 0.0177$ and $R^2 = 0.9956$, while UV-280 with a straight line in the equation of $y = 0.8915x + 0.0481$ and $R^2 = 0.7956$. This shows that these three methods have a very significant relationship between total protein and the method itself. However, when putting into consideration the R^2 values, it can be observed that UV-280 has a low significance than the Bradford and Lowry methods.

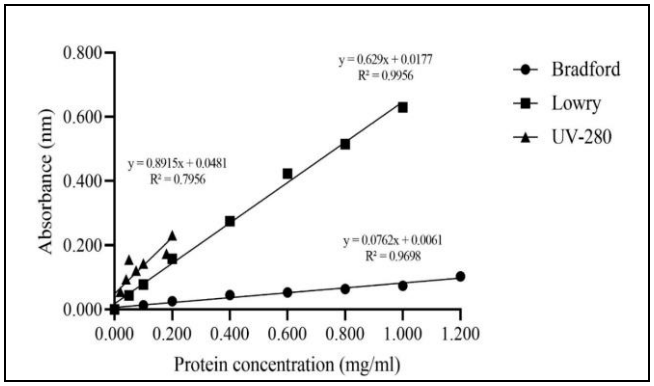

Fig 3: Standard calibration curve plotted for Bradford, absorbance at λ=595 nm, Lowry, absorbance at λ=660 nm and UV280, absorbance at λ=280 nm.

Observation and comparison of protein contents with different methods for C. *vespertilionis* leaves samples (GS, GP, RS and RP) is presented in Table 1 and Figure 2, respectively. Consequently, the highest protein content among the samples was shown in the Bradford method, as compared to Lowry and UV-280. The Bradford method showed the highest sensitivity for proteins in all the samples with the highest protein content in GP at 4.509 ± 0.040 mg/ml and the lowest in RS (0.528 ± 0.118 mg/ml). Meanwhile, the Lowry and UV-280 method showed almost a similar sensitivity for proteins. Lowry showed sensitivity for proteins at a range of 0.528 ± 0.005 to 0.045 ± 0.014 mg/ml with the highest in GP and the lowest in RS. UV-280 method in the other hand, indicated the lowest sensitivity for proteins with RS at 0.013 ± 0.008 mg/ml and the highest with GP at 0.293 ± 0.003 mg/ml.

Table 1: Protein content (mg/ml ± SD) of C. *vespertilionis* leaves in different methods

Sample	Protein content (mg/ml)		
	Bradford	**Lowry**	**UV-280**
GS	0.637 ± 0.095	0.059 ± 0.012	0.021 ± 0.007
GP	4.509 ± 0.040	0.528 ± 0.005	0.293 ± 0.003
RS	0.528 ± 0.118	0.045 ± 0.014	0.013 ± 0.008
RP	2.632 ± 0.087	0.300 ± 0.011	0.161 ± 0.006

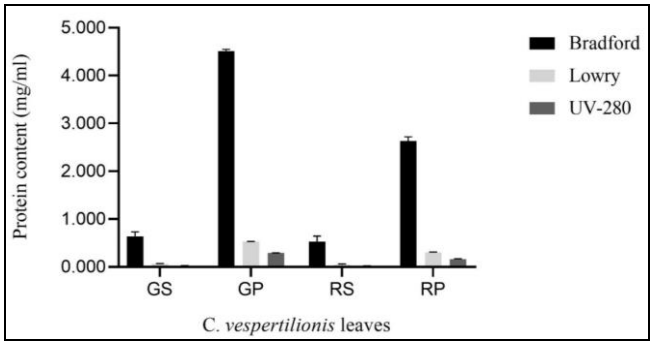

Fig 4: Protein content of C. *vespertilionis* leaves in Bradford, Lowry and UV-280 method.

Based on the sample, it was revealed that GP with the Bradford method obtained high protein contents than Lowry and UV-280 methods. Similar results were achieved from other studies, stating that Bradford method is an accurate method to determine protein contents due to its high sensitivity to proteins and its ability to detect easily with a high molecular weight of proteins inside the samples [12, 13]. With regards to the Bradford method, Coomassie Brilliant Blue G-250 that has been used on this method has a less interaction with other molecules other than proteins and it has a high sensitivity to proteins [8]. Lowry method in this study revealed that the range of total protein contents is not wide and its creation of a uniform response to samples due to Folin-Ciocalteau, does not only react to amino acids tyrosine, tryptophan, cysteine, asparagine and histidine, but is also affected by each tetra unit of peptides in the proteins itself [12, 14, 15]. Folin-Ciacalteu also reacts well with reducing substances such as polyphenols and carbohydrates but is less react to Cu+, which makes the attraction to proteins degrading [8]. UV-280 is the lowest sensitivity to proteins among all the three methods, due to the inability of the amino acid composition in the samples, in reacting with tyrosine [12, 16].

Conclusion

Highest protein content was found from Bradford method particularly in sample GP, and this is due to its high sensitivity to proteins. Conclusively, it can be inferred that the Bradford method is the most accurate, precise, easy, quick and simple method to determine protein content, as compared to Lowry and UV-280.

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References

1. Lee JJ, Yazan LS, Kassim NK, Che Abdullah CA, Esa N, Lim PC et al. Cytotoxic activity of *Christia vespertilionis* root and leaf extracts and fractions against breast cancer cell lines. Molecules,2020:25(2610):1-18.
2. Murugesu S, Perumal V, Balan T, Fatinanthan S, Khatib A, Arifin NJ et al. The investigation of antioxidant and antidiabetic activities of *Christia vespertilionis* leaves extracts. South African Journal of Botany,2020:133:227-235.
3. Upadhyay HC, Sisodia BS, Cheema HS, Agrawal J, Pal A. Novel antiplasmodial agents from *Christia vespertilionis*. Natural Products Communications,2013:8(11):1591-1594.
4. Hofer D, Schwach G, Tabrizi-Wizsy NG, Sadjak A, Sturm S, Stuppner Het al. *Christia vespertilionis* plant extracts as novel antiproliferative agent against human neuroendocrine tumor cells. Oncol Rep,2013:6(29):2219-2226.
5. Garnock-Jones PJ. Note on some folklore drugs and remedies of the Lau group, Fiji. South Pacific Journal of Natural Science,1983:4:4-8.
6. Whiting PA. Commercial production of *Christia subcordata Moench* by establishing cultural practices and by applying plant growth regulators. [Dissertation]. USA: University of Georgia, 2007.
7. Pouplin JN, Tarn H, Tarn H, Phan TA, Dolecek C, Farrar J et al. Antimalarial and cytotoxic activities of ethno pharmacologically selected medicinal plants from South Vietnam. Journal of Ethnopharmacology,2007:109:417-427.
8. Pavel C-L, Mărghitaş LA, Bonta V, Mihai CM, Tomoş LI. Determination of total protein content in royal jelly: a comparison of the Kjeldahl, the Bradford and the Lowry methods. University of Agricultural Sciences and Veterinary Medicine Iasi,2013:59:209-212.
9. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anaytical Biochemistry,1976:72:248-254.
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry,1951:193(1):265-275.
11. Kirschenbaum DM. Molar absorptivity and A1%/1 cm value for proteins at selected wavelengths of the ultraviolet and visible regions. Analytical Biochemistry,1975:68:465-484.
12. Kamizake NKK, Gonçalves MM, Zaia CTBV, Zaia DAM. Determination of total proteins in cow milk powder samples: a comparative study between the Kjeldahl method and spectrophotometric methods. Journal of Food Composition and Analysis,2003:16:507-516.
13. Marshall T, Williams KM. Total protein determination in urine: elimination of a differential response between the Coomassie blue and pyrogallol red protein dye-binding assays. Clinical Chemistry,2000:46:392-398.
14. Chou S, Goldstein A. Chromogenic groupings in the Lowry protein determination. Biochemical Journal,1960:75:109-115.
15. Legler G, Müller-Platz CM, Meniges-Hetikamp M, Pflieger G, Jülich E. On the chemical basis of the Lowry protein determination. Analytical Biochemistry,1985:150:278-287.
16. Haschemeyer RH, Haschemeyer AEV. Proteins: a guide to study by physical and chemical methods, New York: A Wiley-Interscience Publication, 1973, 6-7.