Indonesia International Institute for Life Sciences

INTERNSHIP REPORT

EXTRACTION, PHYTOCHEMICAL SCREENING, AND ANTIOXIDANT ASSAY OF *Coriandrum sativum* folium (Coriander Leaves)

STUDY PROGRAM
Pharmacy

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PREFACE

This internship report was done based on the internship program that was done at Indonesia International Institute for Life Sciences (I3L). The internship program is mandatory for the undergraduate student to obtain the bachelor degree by familiarizing students with the working environment. Internships can also help students in gaining knowledge that can not be obtained from lectures or books.

The internship program was done in around 2 months, including the extraction, phytochemical screening, and antioxidant assays. This internship has helped me to learn how to do phytochemical screening and antioxidant assays.

I would like to express my gratitude to Ms. Audrey Amira Crystalia, S.Farm., M.Sc., Apt., that had accepted me as her intern in this project. I also want to thank the laboratory assistant, Ms. Fadillah Putri Patria for her help preparing the material that is needed for the experiment. Lastly, I want to thank Sir Fandi Sutanto, S.Farm., M.Si., Ph.D., Apt., as my field supervisor and Sir Khoe Ulung Gondo Kusumo, S.Si., M.Sc., as my internship supervisor that has helped me in writing this report.

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INDONESIA INTERNATIONAL INSTITUTE FOR LIFE-SCIENCES (I3L)

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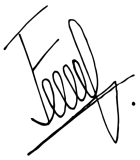

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LIST OF ABBREVIATIONS

CS	*Coriandrum sativum*
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
NaOH	Sodium Hydroxide
PBS	Phosphate buffered saline
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
FRAP	Ferric Reducing Antioxidant Power
TPTZ	2,4,6-Tripyridyl-S-triazine
TPC	Total Phenolic content
TFC	Total Flavonoid Content
IC50	Half maximal inhibitory concentration

SUMMARY/ABSTRACT

Plants are widely used in the medicine field. As a tropical country, Indonesia has a wide variety of plants. One of the plants that can be used are *Coriandrum sativum,* which are found as a promising candidate for improving memory in patients with Alzheimer disease. This study has the aim to do phytochemical screening and antioxidant assays of the CS leaves. The phytochemical screening includes alkaloid, flavonoid, tannin, quinones, saponin, steroid, and terpenoid. Antioxidant assays were also done to determine the IC50 of the extract. The antioxidant assay includes DPPH, ABTS, and FRAP.

CHAPTER 1: INTRODUCTION

1.1. Host Institution/Company

1.1.1. Description about the company

Indonesia International Institute for Life Sciences (I3L) is a life sciences institution that is located in Jakarta. As of 2022, I3L offers several different undergraduate programs in food sciences and nutrition, pharmacy, food technology, biotechnology, bioinformatics, biomedicine, and i3L school of business. I3L has the vision to have an impact on society by using science and innovation to be an interdisciplinary institution that is leading and is connected globally. The mission of I3L is to deliver an life sciences education that are interdisciplinary, multidisciplinary, and also transdisciplinary and at an international level, it is also to support the student entrepreneurship and thinking development in accordance to their disciplines, conduct collaborative with other higher institution, business sector, and government for the research and development activities in life sciences, innovations development in life sciences and implement the innovations to improve the quality of life in Indonesia's, also implementing the tridharma activities by maintaining a continued collaboration with the local and international institution, and government. I3L has 3 values, which are role model, integrity, and grit.

1.1.2. Description of department

Pharmacy bachelor degree in I3L trains students to be able to implement the knowledge in research and clinical application to solve different medical and cosmetic problems. Pharmacy program in I3L has a mission to produce sophisticated pharmacy graduates that are well-versed in current and future pharmaceutical technologies and knowledge, generating research with the focus on the development of next generation pharmaceutical innovation that are top notch at national and international level, and also implementing the pharmaceutical technologies and innovation to solve issues in the community and increasing the health and well-being of the population.

1.1.3. Product of the Host Institution / Company

The I3L Pharmacy program has produced various research results in the form of publications, training, and patents. Graduates from the Pharmacy department of I3L have been accepted to work for various multinational companies. In addition,

through various collaborations with leaders in industry such as pharmaceutics and cosmetics the research in i3L has gone to find functions in those industries.

CHAPTER 2: PROJECT DESCRIPTION

Internship Program

2.1.1. Project Background

Plants have been widely used as traditional medicine. As a tropical country, there are a wide variety of plants in Indonesia. Phytochemicals that are contained in the plant are different for every plant. There are several different phytochemicals, such as alkaloid, flavonoid, saponin, and tannin. *Coriandrum sativum* (CS) also known as coriander are an example of the plant that can be used as a traditional medicine. CS is a part of Apiaceae family whose leaves are usually used for food and medicine

CS leaves contain different types of phytochemicals, such as alkaloid, saponin, and terpenoid. CS leaves and roots have an aromatic flavor and it is often used in soups. CS can be used as an anti-inflammatory, antibacterial agent, and analgesic (Asgarpanah & Kazemivash, 2012). Part of CS can also be used for alleviating spasm, bronchitis, gastric complaint, giddiness, and gout (Tang et al, 2013). CS can also be used for improving memory. It is discovered that the inhalation of volatile oil that is made from CS was found to increase the anxiolytic-antidepressant-like behaviors and decrease the oxidative status in rat models. *Coriandrum sativum* leaves were also found to be a promising candidate for improving memory in patients with Alzheimer's disease (Manuha, 2018).

2.1.2. Scope of the project

The scope of study included in this internship project are the extraction of CS, phytochemical screening , antioxidant assay, and the CS extract total phenolic content and total flavonoid content determination.

2.1.3. Objectives / Aims

The objective of this project is to do the CS extraction, characterize the phytochemical, antioxidant activity of CS leaves and determine the total phenolic content and total flavonoid content.

2.1.4. Problem formulation and Proposed Solutions

Several methods were conducted to further investigate the problem, such as doing the extraction of the CS, phytochemical screening of the CS, and antioxidant assay of the CS. Hence, all the methods done for this internship project are listed below.

2.1.4.1. Extraction of CS leaves

Fresh CS leaves were dried in the oven at 40°C until dry. The simplisia were made into powder by a blender. The method for the extraction was 3 times maceration method by using ethanol 80%. Every 100 g of the simplisia was mixed with 500 ml ethanol 80%. The extract was placed in an erlenmeyer flask and shaken for 24 hours inside the shaker. After the extract was shaken for 24 hours, the solution was filtered by using buncher filtration and whatman no. 1 filter paper. The filtered solution was then placed in the rotary evaporator until the alcohol evaporated. The solution was then placed in a water bath until the ethanol was fully evaporated. The extract was then stored in an amber bottle and stored in the fridge for analysis.

2.1.4.2. Phytochemical screening of CS leaves extract

Alkaloid

The presence of alkaloid was tested using dragendorff reagent. A total of 0.5 gram extract was mixed with 1 ml 2N HCl and 9 ml water and then placed in a 100°C water bath for 5 minutes. After the extract has cooled down to room temperature, the extract was then filtered using whatman no.1 filter paper. The filtrate was then added with the reagent. The appearance of brownish or blackish precipitate indicates the presence of alkaloid.

Flavonoid

The presence of flavonoid was tested using NaOH. NaOH was added to 2 ml of the extract and if there is an appearance of yellow color, it indicates the presence of flavonoid. If flavonoid is present and the solution was added with H_2SO_4 or HCl, it will be colorless.

Saponin

Presence of saponin was tested by mixing the total of 0.5 gram extract with 10 ml hot water inside a falcon tube. The falcon tube was then shaken vigorously for 10 seconds. Appearance of foam with a height between 1-10 cm may indicate the presence of

saponin. A drop of 2N HCL was added and if the foam remains, it indicates the presence of saponin.

Quinones

Presence of quinones was tested by mixing 0.1 gram of the extract in 1 ml water. Few drops of the extract was placed into the test tube and added with 2 ml of concentrated HCl. The appearance of yellowish precipitate indicates that quinones are present in the extract.

Tannin

The presence of tannin in the extract was tested by mixing 1 ml of the extract with 2 ml of 5% ferric chloride solution. Deep green, blue black, or brownish green coloration indicates the presence of tannin.

Steroid and Terpenoid

Salkowski test and Liebermann-Burchard test was used to detect the presence of steroid and terpenoid in the extract. The extract was dissolved in 10 ml chloroform and filtered. The extract was then added with H_2SO_4 until it forms a layer. If there is an appearance of reddish brown coloration in the interface indicates the presence of a terpenoid. The other 5 ml of the extract was mixed with 1 ml of acetic acid anhydride and 1 ml of H_2SO_4 down the side of the tube until it forms a layer. Green-blue coloration indicates the presence of steroids, while pink or violet coloration indicate the presence of terpenoids.

2.1.4.3. Antioxidant assay of *Coriandrum sativum*

FRAP

FRAP reagent was prepared by mixing 3.6 pH acetate buffer, $FeCl_3$ solution with molarity of 20 mM, and 10 mM TPTZ in 40 mM HCl. The standard solution for FRAP assay is trolox. Trolox was prepared with the concentration of 200, 150, 100, 50, and 10 ppm. The extract was then prepared by mixing 5 ml of the CS extract and 5 ml of water. The extract and standard were then mixed with the

FRAP reagent in the 96 well plate with 5 µl of the extract and standard and 150 µl of the reagent. The 96 well plate was then covered by using aluminum foil in the dark for 10 minutes. The absorbance was then read at 593 nm by using plate reader.

ABTS

ABTS solution was prepared by mixing the ammonium persulfate with ABTS. The solution was incubated in the darkness at room temperature for 16 hours. ABTS solution was then diluted into 0.7 absorbance in PBS solution in 734 nm. The standard solution for ABTS assay is trolox. Trolox was prepared with the concentration of 200, 150, 100, 50, 10, and 1 ppm. The extract was prepared in 500 ppm concentration by mixing 10 mg of the CS extract with 20 ml of water. The solution was then diluted with water to make extract with concentration of 400, 300, 200, 150, and 100 ppm. The extract, PBS, and ABTS solution was then placed into 96 well plates with 50 µg extract, 20 µg PBS, and 30 µg of ABTS solution. The absorbance was then read at 734 nm by using plate reader. The IC50 was then calculated by using graphpad.

DPPH

Stock solution of extract was prepared by mixing 100 mg extract with 10 ml methanol to make the stock solution of 10000 ppm. Serial dilution was done to prepare the solution with the concentration of 5000, 2500, 2000, 1500, 1000, 700, 600, 500, 250, 100, 50, and 5 ppm. Standard solution of ascorbic acid was also prepared by mixing 2 mg of the ascorbic acid with 20 ml methanol to make the ascorbic acid with 100 ppm concentration. Serial dilution was also done to prepare the ascorbic acid solution with the concentration of 80, 60, 40, 20, 10, 9, 8, 7, 6, 5, 4, and 3 ppm. The extract and the ascorbic acid was then placed in 96 well plate and mixed with the DPPH solution with the ratio of 1:1. The 96 well plate was then incubated for 30 minutes in a dark room. The absorbance was then read at 517 nm by using plate reader. The IC50 was then calculated by using graphpad.

2.1.4.4. TPC and TFC

TPC (Total Phenolic Content)

Folin-ciocalteu reagent was used to determine the total phenolic compound of *CS* extract. The standard that is used for the phenolic compound determination was gallic acid. Gallic acid was prepared in concentrations of 5, 15, 30, 40, 50, 75, and 100 ppm. The extract was prepared by mixing 25 ml of methanol with 0.2 grams of the extract. The extract solution was then stirred by using a magnetic stirrer. The solution was then filtered by using whatman no.1 filter paper and placed into a 25 ml volumetric flask. Methanol was then added to the volumetric flask until the volume reached 25 ml. The total of 1 ml of the extract and 1 ml of gallic acid was then placed into the test tube. Then 5 ml of 7.5% Folin-ciocalteu reagent was added into the extract and gallic acid. After 8 minutes, 1% NaOH with a total of 4 ml was added into the test tube and the solution was placed in 96 well plates. The absorbance was read at 730 nm by using plate reader.

TFC (Total Flavonoid Content)

Quercetin is used as the standard to determine the total flavonoid content of *CS* extract. Quercetin was prepared by mixing 5 mg of quercetin powder with 100 ml ethanol to make the quercetin with concentration of 5 ppm. The 5 ppm quercetin was then diluted using ethanol into quercetin with concentration of 4, 3, 2, and 1. The extract with the concentration of 2 ppm was prepared by mixing 25 ml of methanol with 0.05 grams of the extract. The extract was stirred by using a magnetic stirrer. The solution was then filtered by using whatman no.1 filter paper into a 25 ml volumetric flask. Ethanol was then added into the volumetric flask until the volume reached 25 ml. 1 ml of the extract and standard was then mixed with 0.1 ml 1M CH_3COOK, 0.1 ml of 10% $AlCl_3$, and 2.8 ml water. The solution was then placed into 96 well plates and incubated at room temperature for 30 minutes. The absorbance was then read at 415 nm by using plate reader.

CHAPTER 3: FINDINGS

3.1. Results

Extraction

The total of 20.5 kg of fresh CS leaves was dried which yielded 213.02 gram simplisia. The simplisia were then extracted by using a 3 times maceration method. The extract obtained is 25.2 gram. The yield can be calculated by using following equation:

$$\%yield = \frac{Extract\ weight}{Dried\ coriander\ weight} x100\%$$

By using the equation, the yield for the *CS* compared to the dried leaves weight is 11.83%.

Phytochemical Screening

Phytochemical screening was conducted and the results were listed in **Table 1.** According to the result, the *Coriandrum sativum* leaves contain saponin and tannin, while alkaloid, flavonoid, quinones, steroid, and terpenoid are not present in the sample.

Table 1. Phytochemical result of *Coriandrum sativum* extract

No.	Phytochemical	+/-*
1	Alkaloid	-
2	Flavonoid	-
3	Saponin	+
4	Quinones	-
5	Tannin	+
6	Steroid	-
7	Terpenoid	-

* + indicates the presence of the phytochemical
* - indicates the phytochemical is not present in the extract

FRAP

Trolox was used as the standard curve to calculate the FRAP value. The standard curve was then made and the equations are used to determine the concentration of the extract. The concentration of the extract found from the standard curve is 12.2 ± 0.02 mg TE/gr.

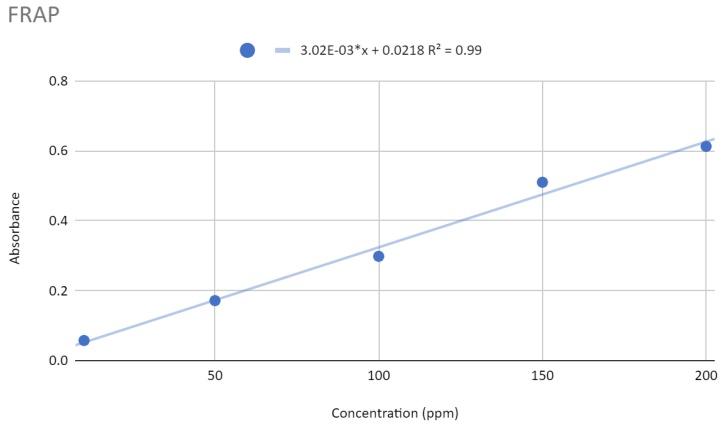


Figure 1. Standard curve of Trolox

ABTS

ABTS was done by using trolox as the standard. The percent inhibition of trolox and the CS was then made into graphs. The IC50 of ABTS assay of CS is 47.02 ± 8.56 ppm.

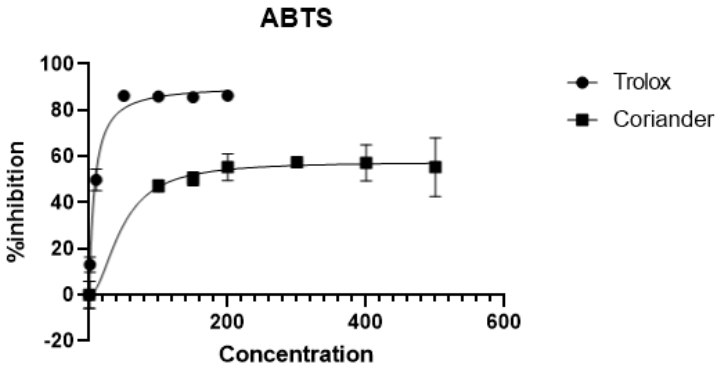


Figure 2. Percent inhibition of Trolox and *Coriandrum sativum*

DPPH

DPPH was done by using ascorbic acid as the standard. The percent inhibition of ascorbic acid and CS was then made into graphs. The IC50 of DPPH assay of *CS* is 1889 ± 123.7 ppm.

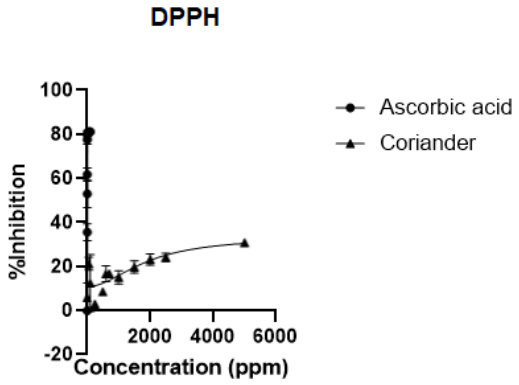


Figure 3. Percent inhibition of Ascorbic acid and *Coriandrum sativum*

TPC

Gallic acid was used as the standard to calculate the TPC. The standard curve was then made and the equations are used to determine the concentration of the extract.

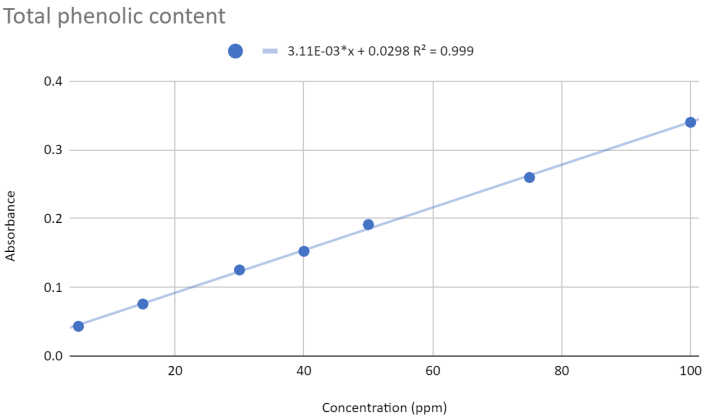


Figure 4. Standard curve of Gallic acid

From **Figure 4,** the standard curve has good linearity because the r is 0.999 and it is close with 1. The concentration of the extract found from the standard curve is 160.85 ppm. Following equation was then used to calculate the total phenolic content:

$$TPC = \frac{extract\ concentration\ x\ volume\ of\ extract}{weight\ of\ extract x 1000}$$

The total phenolic content found in the extract was 20.1 ± 0.007 mg GAE/gr when calculated by using the equation.

TFC

Quercetin was used as the standard to calculate the TFC. The standard curve was then made and the equations are used to determine the concentration of the extract.

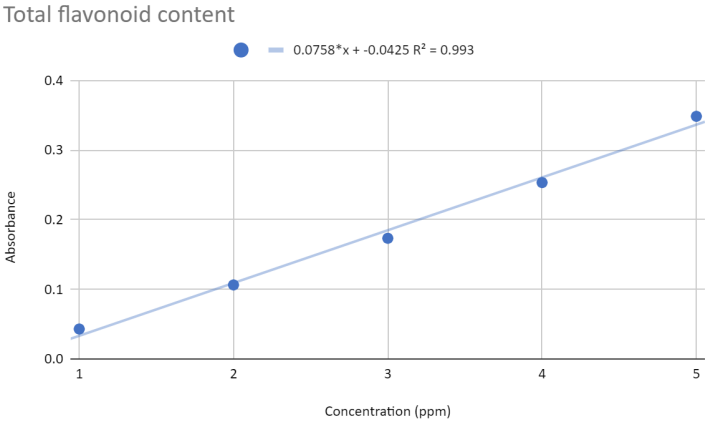


Figure 5. Standard curve of Quercetin

From **Figure 5,** the standard curve has good linearity because the r is 0.993 and it is close with 1. The concentration of the extract found from the standard curve is 5.7 ppm. Following equation was then used to calculate the total phenolic content:

$$TFC = \frac{extract\ concentration\ x\ volume\ of\ extract}{weight\ of\ extract x 1000}$$

The total flavonoid content found in the extract was 2.85 ± 0.0156 mg QE/gr when calculated by using the equation.

3.2. Discussion

2.1. Extraction

Based on the study conducted by Jangra et al (2018), the yield of *Coriandrum sativum* leaves extraction using ethanol is 3.35%, while in this research, the yield of the *Coriandrum sativum* leaves is 11.83%. The method in the study by Jangra et al (2018) are not specified, but the difference in the extraction yield can be caused by the difference in the method of extraction. In this study, the extract was macerated 3 times, hence the percentage yield in this study is around 3 times more than the percentage yield in the study that is conducted by Jangra et al (2018).

2.2. Phytochemical screening

According to the study conducted by Ashika et al (2018), methanol extract of CS contains tannin and terpenoid, but absence of alkaloid, flavonoid, saponin, and steroids. A study by Yulia et al (2020), methanol extract of CS contains alkaloid, saponin, and terpenoid, but absence of flavonoid and steroid, while tannin is not tested in the study. In this research, the phytochemicals that are detected in the CS are tannin and saponin, while alkaloid, flavonoid, steroid, and terpenoid are absent. The absence of flavonoid in the phytochemical screening can be caused by a low

amount of total flavonoid content in the extract. The difference in the result of phytochemicals of terpenoid can be caused by the color obstruction of the extract in the terpenoid test, while the different result of alkaloid can be caused by the difference of the reagent used in the research. In study by Yulia et al (2020), the reagent used for the alkaloid detection is Mayer's reagent, while in the research the reagent that is used is the dragendorff reagent. The difference can also be caused by the difference in the solvent that is used for the CS extraction and the difference in the growth place. In the study conducted by Yulia et al (2020), the CS leaves are obtained from Sumatera Barat, while in this research, the CS *leaves are* obtained from Lembang.

2.3. Antioxidant assays

FRAP

Ferric reducing antioxidant power (FRAP) assay are used to measure antioxidant power based on the ability to reduce ferric-tripyridyltriazine to ferrous-tripyridyltriazine complex with intense blue color at low pH and in maximum absorbance 593 nm in the presence of TPTZ. FRAP can not detect compounds that act by hydrogen transfer, but FRAP is often used because it is a simple, cost effective and rapid method (Cerretani & Bendini, 2010). Based on a study conducted by Kamiloglu et al (2014), the 80% methanol extract of *Coriandrum sativum* FRAP value was 11.8 ± 0.31 mg TE/gr, while in this research, the FRAP value are 12.2 ± 0.02 mg TE/gr. The difference in the FRAP value can be caused by the difference in the solvent used for the extraction.

ABTS

ABTS assay works based on the interaction between antioxidants and the pre generated ABTS radical cation. ABTS can be easily used to detect quantitatively due to the bleaching of the absorption spectrum at several absorbences, such as 414, 417, 645, 734, and 815 nm (Ilyasov et al, 2020). Based on the study conducted by Nhut et al (2020), the IC50 of CS leaves in ethanolic extract are 54.489 ppm, while in the research, the IC50 of CS leaves are 47.02 ± 8.56 ppm. The difference in the result can be caused by the difference in the concentration of solvent used for the extraction. In the study conducted by Nhut et al (2020), the concentration of ethanol that is used for the extraction is 96% ethanol, while in this research, the concentration of ethanol that is used is 80%. A study conducted by Do et al (2014) stated that antioxidant activity is decreased when the amount of water increases in

organic solvent. Difference in the place of growth can also affect the result of IC50, because in the study conducted by Nhut et al (2020), the CS grows in Vietnam, while in this research, the plant is obtained from Lembang.

DPPH

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical assays are used to measure antioxidant activity that are based on transfer of electrons that produce solution with violet color in the presence of ethanol. The free radical is reduced when the antioxidant molecules are present. DPPH assay is an easy and rapid way to evaluate antioxidant activity (Garcia et al, 2012). Based on a study conducted by Tang et al (2013), the IC50 of CS leaves in aqueous extract are 1335 ± 37.7 ppm, while in the research, the IC50 of CS leaves are 1889 ± 123.7 ppm. The difference in the result can be caused by the difference in the solvent for the extraction. A study conducted by Do et al (2014) stated that antioxidant activity is decreased when the amount of water increases in organic solvent.

2.4. Total Phenolic and Flavonoid Content

TPC

Total phenolic content (TPC) was done by using folin-ciocalteu reagent. Folin-ciocalteu reagents are a method that are based on electron transfer and give reducing capacity that are expressed as total phenolic content (Noreen et al, 2017) . Based on the study by Nasution & Arifah (2019), the total phenolic content of *CS* leaves extracted in 96% ethanol are 30.7049 mg GAE/gr, while in this research the total phenolic content is 20.1 ± 0.007 mg GAE/gr. The difference in the result of the total phenolic content can be caused by the difference in the concentration of ethanol, as in this research, the concentration of ethanol used is 80%. Based on the study conducted by Yusof et al (2020), total phenolic content increases when the concentration of ethanol increases.

TFC

Total flavonoid content (TFC) was used to estimate the amount of flavonoid in the extract. The aluminum chloride will form an acid stable complex with the C4 keto group and between C3 or C5 hydroxyl group of the flavones and flavonols (Ahmed & Iqbal, 2018). Based on the study by Sadoun et al (2021), the total flavonoid content of CS leaves extracted in ethanol for 24 hours are 8.112 ± 0.115 mg QE/gr, while in this research the total flavonoid content is 2.85 ± 0.016 mg QE/gr. The difference in

the result can be caused by the difference in the ethanol concentration. The concentration of ethanol in this research is 80% and the study conducted by Yusof et al (2020) stated that the total flavonoid content increases when the concentration of ethanol increases. The place of growth can also affect the total flavonoid content, as in the study conducted by Sadoun et al (2021), the plant grows in Iraq, while in this research the plant was obtained from Lembang.

CHAPTER 4: CONCLUSION AND RECOMMENDATIONS

In conclusion, the CS yield from the maceration process is 11.83%. CS extracted in this research was found to contain saponin and tannin. The FRAP result found was 12.2 ± 0.02 mg TE/gr. The IC50 found from the ABTS assay is 47.02 ± 8.56 ppm, while the IC50 from the DPPH assay are 1889 ± 123.7 ppm. The result of total phenolic content was found was 20.1 ± 0.007 mg GAE/gr, while the total flavonoid content was 2.85 ± 0.015 mg QE/gr.

The phytochemical screening of steroid and terpenoid is considered a non suitable for the crude extract because of color obstruction. In the future research, the CS extract can be diluted or purified first so there is less risk of color obstruction.

CHAPTER 5: SELF REFLECTION

This internship was done during COVID-19 period where students still need to follow strict health protocol in I3L. It is still uncomfortable to use masks every time, but the masks are needed to reduce the risk of being infected by COVID-19. I gained much knowledge from this project alone such as good time management skills.

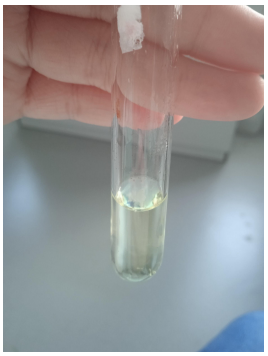
During this internship, I worked in the extraction process, phytochemical screening, and antioxidant assays alone in the laboratory. I got help from many friends and juniors in working on this project and I feel grateful for that. I am also scared of making mistakes and causing the experiment to fail, however I can also learn by making mistakes. In addition, I have also learned that each problem is unique and the solutions to every problem should be approached carefully and in a methodological manner as can be seen from the results of the qualitative experiment which is unsuitable for this extract. Through grit and curiosity we continued to do the quantitative experiment and learned more about the extract. Hence the experience at working on this project has taught me to be more careful in working and helped me to be more confident in my ability which I hope will help me through life.

APPENDICES

Appendix 1. CS phytochemical screening result

(1) Alkaloid, (2) Flavonoid, (3) Saponin, (4) Quinones, (5) Tannin, (6) Steroid, (7) Terpenoid

(1)



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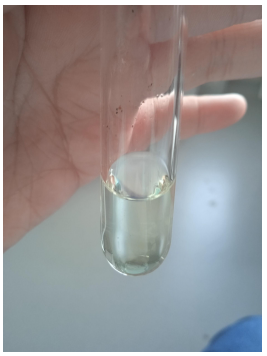

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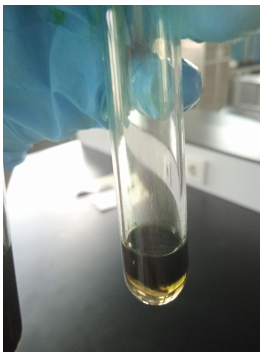

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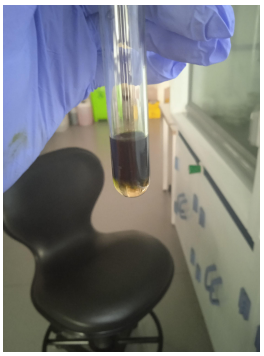

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