Indonesia International Institute for Life Sciences

INTERNSHIP REPORT

Phytochemical Characterization of *Marchantia paleacea, Pogonatum neesii,* and *Litsea Oppositifolia* Ethanolic Extract

STUDY PROGRAM
Pharmacy

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PREFACE

This internship report was made to fulfil the requirements for the credited internship program. The title of this project is "Phytochemical Characterization of *Marchantia paleacea, Pogonatum neesii,* and *Litsea Oppositifolia Extract.*" This research period up to the writing of this report was done during August to September 2022 in i3L.

I would like to express my deepest gratitude first of all to God who helped me throughout every process and enabled me to finish my project and this report. I also would like to extend my gratitude towards my field supervisor apt. Pietradewi Hartrianti, M.Farm., Ph.D who guided me throughout these two months, apt. Fandi Sutanto, S.Farm., M.Si., Ph.D. who helped me in using the LC-MS and in processing the data, and also Dr. Khoe Ulung Gondo Kusumo, S.Si., M.Sc. who guided me in writing this report. To Ko Gio who helped me in the beginning of using the LC-MS even though he didn't have to, thank you very much. To Vivian, Jason, Crishella, and Sun Joshua who helped me do my phytochemical assays, also to Fenny and Glen who helped me in the end, thank you. I would also like to say thank you to my peers and Ci Erika who supported me throughout this project.

Jakarta, Indonesia, 6 October 2022

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Phytochemical Characterization of *Marchantia paleacea, Pogonatum neesii,* and *Litsea Oppositifolia* Ethanolic Extract

This internship was done at Indonesia International Institute for Life-Sciences (i3L)

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LIST OF ABBREVIATIONS

AE	: Atropine equivalent
BCG	: Bromocresol green
GAE	: Gallic acid equivalent
LO	: *Litsea oppositifolia* extract
NMR	: Nuclear Magnetic Resonance
LC-MS	: Liquid chromatography - Mass spectrophotometry
MP	: *Marchantia paleacea*
MW	: Molecular weight
PN	: *Pogonatum neesii*
UV-Vis	: UV- Visible
ROS	: Reactive oxygen species
QE	: Quercetin equivalent

SUMMARY

i3L is a life science higher education and research institute in Jakarta, Indonesia. Their Pharmacy department in the School for Life Sciences provides research project opportunities for students. This project aims to do phytochemical characterization of *Marchantia paleacea, Pogonatum neesii*, and *Litsea oppositifolia* extracts as a preliminary study for two bigger projects that aims to investigate anti-cancer and anti-aging properties of these extracts. To achieve this aim, phytochemical screening, assay, and LC-MS analysis was conducted. The phytochemical screening and assay shows the presence of flavonoid in *Marchantia paleacea* and *Litsea oppositifolia* extract, phenolic content in all three extracts, and a small concentration of alkaloids in all extracts. No extract showed the presence of saponins. Out of the three quantified functional groups, phenolics were found to be the most prominent secondary metabolite in all three extracts. LC-MS analysis revealed the presence of eugenol in *Litsea oppositifolia* extract. Further analysis using standards or NMR analysis could be done to further elucidate the components contained in the extracts.

Keywords: phytochemical characterization, phytochemical screening, total phenolic content, total alkaloid content, total flavonoid content, LC-MS

CHAPTER 1: INTRODUCTION

1.1. Host Institution / Company

1.1.1 Description about the company

Indonesia International Institute for Life Sciences (i3L) is a premium life science higher education and research institute located in Pulomas, Jakarta, Indonesia established in 2014 as a collaboration between KALBE Educational Foundation in Indonesia and Karolinska Institute and Swedish University of Agricultural Sciences in Sweden. Today, i3L offers 10 undergraduate programs, 6 of which are under the school of life sciences (Bioinformatics, Biomedicine, Biotechnology, Food Science and Nutrition, Food technology, and Pharmacy). i3L's vision is to be a leading and globally connected interdisciplinary institution that impacts society through science and innovation. Their missions are : (1) To deliver an inter-, multi-, and transdisciplinary education in life sciences at an international level, as well as to support the development of our students' scientific and entrepreneurship thinking in accordance to their disciplines. (2) To conduct collaborative research and development activities in life sciences and business with other higher education institutions, the business sector, and the government. (3) To develop innovations in life sciences and to implement and improve Indonesia's quality of life. (4) To maintain a continued collaboration with the government and both local and international higher education institutions in order to implement the Tridharma activities.

1.1.2 Description of Department

Pharmacy is one of the departments in the School of Life Science. The pharmacy program in i3L is focused on medicine, which covers drug discovery, development, clinical trial, drug delivery, drug management and also patient counselling.

1.1.3 Product of the Host Institution / Company

Pharmacy study program provides research project opportunities for students. This particular internship is part of two projects. One project is a collaboration between pharmacy, biomedicine, and biotechnology study programs with Universitas Indonesia which aims to check for anti-aging properties in *Litsea oppositifolia* ethanolic extract. The other project is a collaboration between pharmacy, biomedicine, and biotechnology study programs with BRIN Cibodas check for anti-cancer properties of *Marchantia paleacea* and *Pogonatum neesii*.

CHAPTER 2: PROJECT DESCRIPTION

2.1. Internship Project

2.1.1. Project Background

Medicinal plants have been used in traditional medicine to cure diseases for decades due to their active biological properties (Morales et al., 2017; WHO, 1993). Medicinal plants also play a significant role in the process of new pharmacologically active compound development (Viera et al., 2014). Among many biological properties, two that are often investigated by researchers are anti-cancer and anti-aging properties, which are exhibited by several secondary metabolite groups (Cragg & Newman, 2005). Several secondary metabolite groups known to exhibit great anticancer potential are alkaloids, diterpenes, terpenes, and polyphenolics (Seca et al., 2018). On the other hand, secondary metabolites that exhibit anti-aging properties usually are antioxidants which could scavenge reactive oxygen species (ROS). The main group of secondary metabolites that exhibit this property is polyphenols (Zhang et al., 2015).

The existence of diverse medicinal plants are found in Indonesia, which is recognized as one of the most biodiverse countries (Tambunan et al., 2017; Dirzo & Raven, 2003). Accordingly, there are many plant species to discover and explore for their biological potential. Of the many, *Pogonatum neesii* (PN), *Marchantia paleacea* (MP), and *Litsea oppositifolia* (LO) are three plant species native to Indonesia with minimal research on their pharmacological significance (Siregar et al., 2013; Crosby & Magill 2022). Two of which are bryophytes, PN and MP, which are non-vascular land plants known to synthesise a wide variety of secondary metabolites. Several studies have shown that bryophyte extracts and metabolites such as phenolics exhibit anti-cancer properties (Ciaanciullo et al., 2022; Asakawa, 2007). LO is a species in the *Litsea* genus, which is the largest genus in the *Lauraceae* family. Several studies have shown that some species in the genus of *Litsea* possess high amounts of polyphenols, and therefore exhibit anti-aging properties (César et al., 2022; Dalimunthe et al., 2021).

Therefore, it is necessary to conduct phytochemical screening and quantification of potential biologically active functional groups in PN, MP, and LO known to exhibit anti-cancer and anti-aging properties, allowing future research to be conducted based on their phytochemical constituents.

2.1.2. Scope of the Project

The scope of this project is to do phytochemical screening, assay, and LC-MS to identify compounds contained in MP, PN, and LO ethanolic extracts. The phytochemical screening

includes screening for flavonoid, tannin/phenolic, alkaloid, and saponin. The phytochemical assay includes total flavonoid content, total phenolic content, and total alkaloid content. The LC-MS analysis is limited to compound libraries available for the genus of each extract, since data for each species were limited.

2.1.3. Objectives

The objective of this project is to investigate the phytochemical content and quantify the total phenolic, alkaloid, and flavonoid content of MP, PN, and LO ethanolic extract.

2.1.4 Problem formulation and proposed solutions

In order to investigate the phytochemical contents of MP, PN, and LO ethanolic extracts, preliminary phytochemical screening, assay, and LC-MS was conducted.

Phytochemical Screening

The phytochemicals screened were flavonoid, tannin, phenolic, alkaloid, and saponin. The crude extract of each plant was first diluted with suitable solvents. 0.1 gr of LO crude extract was dissolved in 50 mL type III water while 0.1 g MP and PN crude extract was dissolved in 50 mL methanol to obtain 2000 ppm extract solutions. The procedure for flavonoid, alkaloid, tannin, and phenol screening was conducted according to a paper by Kancherla et al (2019). For flavonoid screening, an alkaline reagent test was performed, where 2-3 drops of 2N NaOH was added to 2mL of each extract. A positive flavonoid test was indicated by the presence of yellow colour that disappeared upon addition of 2N HCl. Tannin and phenolic screening was performed through a ferric chloride test which was done by taking 1 mL extract in a test tube, added with 1 mL 5% FeCl3. The presence of most tannin and phenolic would be indicated by dark blue-greenish black colour. Alkaloid screening was performed through Mayer's reagent for alkaloid screening, which was freshly prepared by combining 0.68 grams of mercury chloride and 2.5 grams of potassium iodide in 50 mL water. 2 mL of the Mayer's reagent along with 5 mL ethanol was added to 2 mL plant extract. The formation of greenish or creamy colour precipitate indicates a positive alkaloid response. Saponin screening was performed based on the method by Jayapriya & Shoba (2014) with a few modifications. 2 mL of the plant extract was added into a 15 mL falcon tube. 1 mL hot water was added and the tube was shaken vigorously for 10 seconds. The foam formed was measured initially and after 10 minutes. Then, 1 drop of 2N HCl was added. A positive saponin content was indicated by the presence of a stable foam throughout the test.

Total Flavonoid Content

The method for quantification of total flavonoid content of each extract was taken from the second edition of Farmakope Herbal Indonesia (2017) with several adjustments. 0.08 grams of crude extract were diluted in 10 mL ethanol and then filtered. After which, the volume was adjusted to 10 mL using ethanol. Standard curve was made using quercetin in ethanol at concentrations of 25, 50, 75, 100, and 125 µg/mL. 0.5 mL sample solution and standard concentration was each pipetted into a test tube. Then, 1.5 mL ethanol, 0.1 mL aluminium chloride 10%, 0.1 mL of 1 M sodium acetate, and 2.8 mL type III water was added and mixed. The solution was then left to incubate for 30 minutes at room temperature. Absorbance measurement using UV-Vis spectrophotometer was done at 415 nm against aluminium chloride blank. PN and MP samples were diluted by 1 in 6 using ethanol prior to absorbance measurement to ensure the absorbance value was within linearity range.

The resulting absorbance values were calculated using the equation generated from the standard curve, and then converted into mg Quercetin Equivalents (QE) using the following equation:

$$QE = \frac{C \, x \, V}{W \, x \, 1000}$$

C is the concentration of the extract calculated from the standard curve in ppm, V is the volume of solvent used to dilute the crude extract in mL, while W is the weight of the crude extract diluted in grams.

Total Phenolic Content

Total phenolic content quantification was conducted based on the second edition of Farmakope Herbal Indonesia (2017) with several modifications. For extract preparation, 0.08 grams of each extract was dissolved in 10 mL methanol and then filtered. The resulting volume was then adjusted to 10 mL with methanol. Standard curve was made using gallic acid in methanol at concentrations of 5, 15, 30, 50, 70, and 100 µg/mL. 0.5 mL of sample solution and standard concentration was each pipetted into a test tube. Then, 2.5 mL folin-ciocalteu 7.5% in water solution was added and left to react for 8 minutes. 2 mL NaOH 1% was added and the solution was incubated for 1 hour at room temperature prior to absorbance measurement using UV-Vis spectrophotometer at 730 nm against folin-ciocalteu blank samples.

The resulting absorbance values were calculated using the equation generated from the standard curve, and then converted into mg Gallic Acid Equivalents (GAE) using the following equation:

$$GAE = \frac{C \, x \, V}{W \, x \, 1000}$$

The letters in the equation represent concentration in ppm, volume in mL, and weight in grams as mentioned in the total flavonoid content method above.

Total Alkaloid Content

Total alkaloid content quantification was conducted according to Mythill et al (2014) with some modifications. Extract preparation was done by dissolving 0.05 gr extract in 25 mL methanol. Bromocresol green (BCG) solution was prepared by heating 34.9 mg BCG with 1.5 mL NaOH 2N and 2.5 mL distilled water until completely dissolved. Then, the solution was diluted to 500 mL with distilled water. Phosphate buffer solution at pH 4.7 was prepared by adjusting the pH of sodium phosphate 2M (7.16 g Na_2HPO_4 in 100 mL distilled water) with citric acid (4.2 g in 100 mL). Atropine standard solution was made by dissolving pure atropine in distilled water at concentrations 20, 40, 60, 80, and 100 µg/mL.

0.5 mL of each dissolved plant extract and standard solution was pipetted into a test tube and was added with 0.5 mL 2N HCl. A 2.5 mL BCG solution was added, along with a 2.5 mL phosphate buffer. The solution was then transferred into a separating funnel and shaken with 0.5, 1, 1.5, and 2 mL chloroform by vigorous shaking. The chloroform was collected in a 10 mL measuring cylinder and diluted to 5 mL with chloroform. The absorbance for the sample and standard solution was measured using UV-Vis spectrophotometer at 470 nm against blank, which is a solution that is not added with BCG.

The resulting absorbance values were calculated using the equation generated from the standard curve, and then converted into mg Atropine Equivalents (AE) using the following equation:

$$AE = \frac{C \, x \, V}{W \, x \, 1000}$$

The letters in the equation represent concentration in ppm, volume in mL, and weight in grams as mentioned in the total flavonoid content method above.

LC-MS

LC-MS analysis was conducted by first searching for the compound library of LO, MP, and PN. Unfortunately, there was limited information regarding the chemical constituent of each species during the time this research was conducted. Hence, the researcher compiled the list of compounds contained in other species with the same genus as the target plants. This final list of compounds obtained for each species along with their molecular weight served as a compound library for the determination of compounds contained in each extract.

To do LC-MS analysis, 0.01 gr of LO extract were dissolved in 10 mL type 1 water for LC-MS and filtered using a 0.01 nylon filter. 0.01 gr of MN and PN extracts were dissolved in 10 mL methanol and also filtered prior to transferring them to LC-MS vials. Each solvent was also filtered and poured into LC-MS vials for blank measurement. The parameters for LC-MS were the following: flow rate of 0.3 ml/min, with mobile phase of methanol:water with 0.1% formic acid (50:50), with a run time of 30 minutes each. The chromatograms along with the mass-spectrum of each extract were observed and compared to their respective blanks and to their compound libraries. Peaks corresponding to the molecular weight in the compound library were highlighted, and their respective structures were investigated for the presence of fragmentation patterns in the mass spectrum. A compound that has a corresponding fragmentation pattern was determined as present in the extract.

CHAPTER 3: FINDINGS

3.1. Results

3.1.1 Phytochemical Screening

Flavonoid phytochemical screening of LO extract shows a yellow colour forming when NaOH 2N was added. The colour faded with the addition of 2N HCl. This result indicated a positive flavonoid content in LO. PN extract did not develop a yellow colour upon NaOH addition, but it did increase in opacity (became turbid). Addition of 2N HCl caused the turbidity to disappear, and a colour change to slightly brown. The flavonoid content of PN was therefore not detected through phytochemical screening. MP extract changed colour to red-dark brown upon 2N NaOH addition. When 2N HCl was added, the colour changed back to the colour of the initial extract. Considering the initial colour of the extract was dark green, the development of dark brown colour could be due to the colour yellow formed.

The phytochemical screening result shows presence of tannin and or phenol in MP and LO as indicated by the darkening of these two solutions to a darker brown colour upon the addition of $FeCl_3$ compared to the control. PN extract did not show a clear colour change, and therefore is considered to give a negative result. The result of alkaloid screening shows slight change in colour of LO extract from light yellow to light green, indicating the presence of alkaloid in the extract. MP and PN diluted extracts were green prior and after the addition of Mayer's reagent with no visible colour change. The saponin froth test shows little frothing in all the extracts upon 10 seconds of shaking. After 10 minutes and the addition of HCl, the foam disappeared. Therefore, saponins were indicated as absent in all three extracts (table 1).

Table 1. Phytochemical Screening Result

	LO extract	PN extract	MP extract
Flavonoid	+	-	+
Tannin and Phenol	+	-	+
Alkaloid	+	-	-
Saponin	-	-	-

3.1.2 Total Flavonoid Content

The standard curve for total flavonoid content shows a coefficient correlation of 0.999. The concentration of flavonoid in each extract was calculated using the quercetin standard curve equation of Y = 0.00314x + 0.0274. The concentration in ppm was then converted into mg QE,

where LO extract contains 0.993 ± 0.401 QE mg/g , PN extract contains 0.064 ± 0.690 QE mg/g, and MP extract contains 14.076 ± 2.663 QE mg/g.

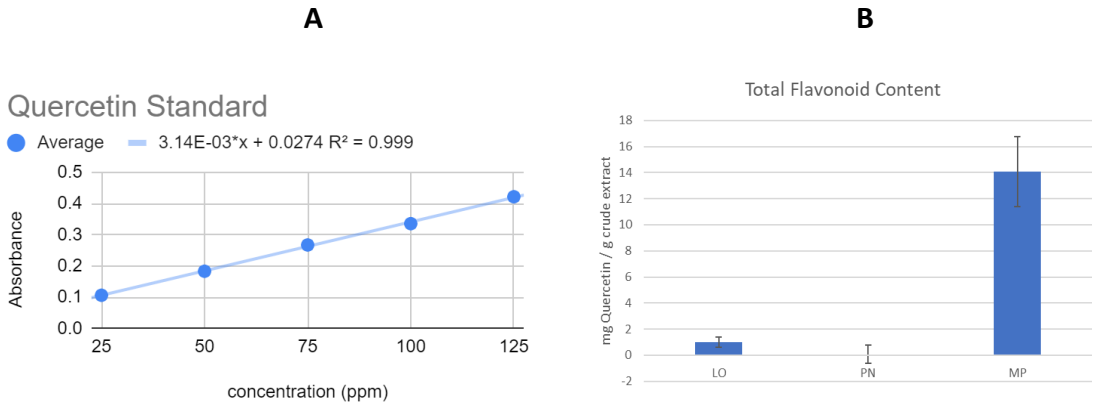


Figure 1. Quercetin standard curve (A) and the Total Flavonoid Content (B).

3.1.3 Total Phenolic Content

The standard curve for total phenolic content shows a coefficient of correlation 0f 0.997. The concentration of total phenol was calculated based on the gallic acid standard curve equation of Y = 0.00732x + 0.0288. All extracts show the presence of phenolics, where MP extract contains the highest phenolic content at 78.572 ± 1.565 GAE mg/g, followed by PN extract at 48.654 ± 1.239 GAE mg/g. LO extract contained the lowest concentration of total phenol at 33.559 ± 0.783 GAE mg/g.

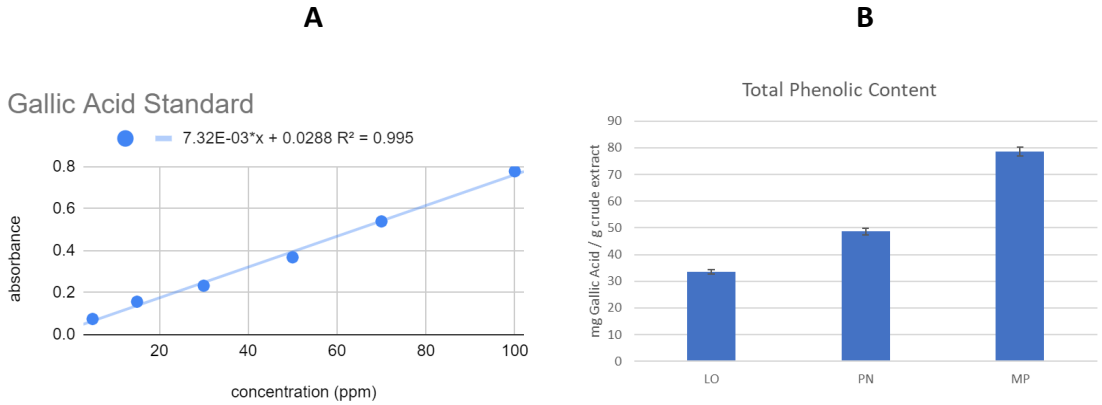


Figure 2. Gallic Acid Standard Curve (A) and the Total Phenolic Content (B).

3.1.4 Total Alkaloid Content

The standard curve for total alkaloid content shows a coefficient of correlation of 0.995. The concentration of total alkaloid content in each extract was calculated using the atropine standard curve equation of Y = 0.00103x + 0.0401. All extracts show a presence of alkaloid

content, where the highest concentration was seen in PN extract with 25.356 ± 11.336 AE mg/g, followed by MP extract at 19.045 ± 3.676 AE mg/g. The lowest concentration of alkaloid content was seen in LO extract with 7.880 ± 1.401 AE mg/g.

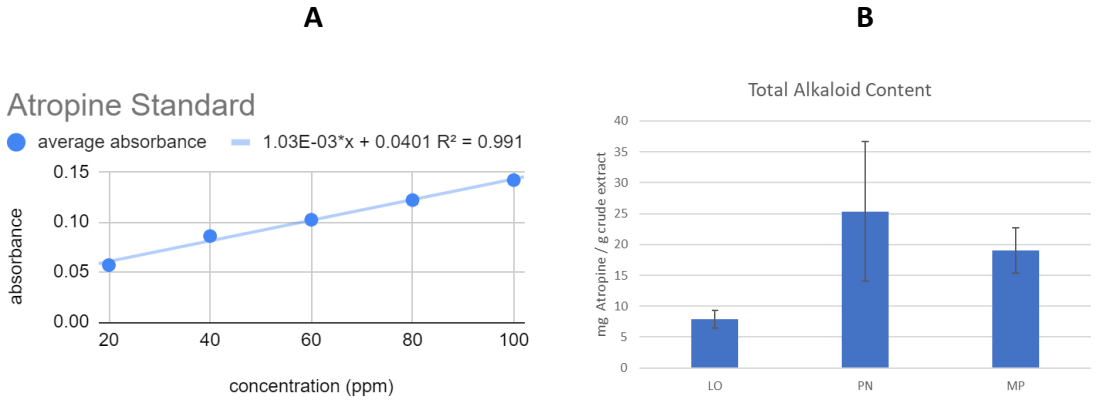


Figure 3. Atropine Standard Curve (A) and Total Alkaloid Content (B).

3.1.5 LC-MS

Compound Library

The source of chemical constituents used for both LO extracts were based on two reviews regarding the chemical constituents of plants from the genus *Litsea* (Agrawal et al., 2011; Kong et al.,2015). Additionally, three other papers that report chemical constituents of *Litsea cubeba* (Kamle et al., 2019), *Litsea Fruticosa* (Liu et al., 2013), and *Litsea lancifolia* (Sulaiman et al., 2011) were also used, as they contained several compounds not listed in the two reviews. Hence there were a total of 164 compounds in the class of alkaloid, flavonoid, sesquiterpene, diterpene, aminde, lignan, steroid, and fatty acid in the list of possible chemical constituents of LO (Appendix 2).

For the list of potential chemical constituents of MN, the list of compounds was based on a review by Jantwal et al (2019) regarding the genus Marchantia, and 4 papers which covered several chemical constituents contained in *Marchantia convoluta* (Chen & Xiao, 2005; Sloan-Kettering, 2006; Cao et al., 2007), and *Marchantia Tosana* (Lahlou et al., 2000). The final list contained 20 compounds from the class of flavonoid, phenol, isoprenoid, sterol, fatty acid, and hydroxybenzaldehyde (Appendix 3).

For PN, there was one paper which elucidated a list of compounds contained in *Pogonatum inflexum* (Duan, 2020). Hence, the list for PN contained 16 compounds in the class of flavonoid and phenolic aldehyde (Appendix 4).

LC-MS Reading

The chromatogram for each extracts and their blank are displayed in **Figure 4** and **Figure 5** below. Out of the 164 compounds in the LO library, 30 compounds with 20 different molecular weights matched with the LO compound library. Out of the 20 different molecular weights, 9 indicated more than 1 compound, which are compounds with molecular weights of 256, 314, 326, 328, 339, 342, 343, 356, and 433 (table 2). The probable compounds were narrowed down by matching the MS fragmentation pattern to the structure of each molecule, probable compounds were identified and marked with a (*) in the table below.

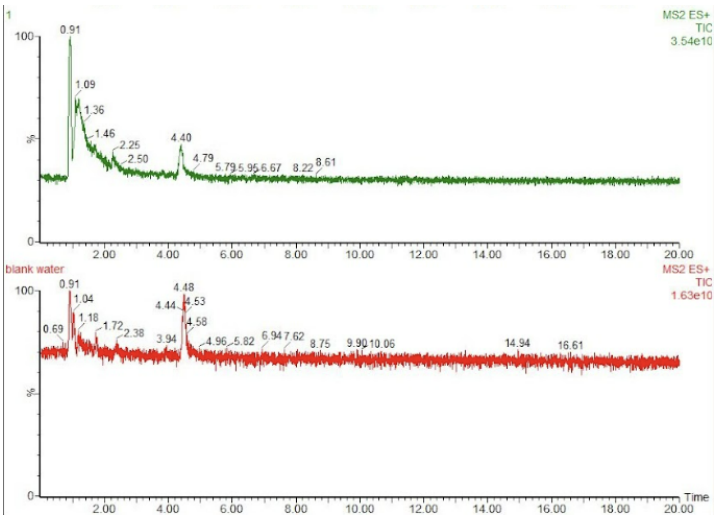


Figure 4. Chromatogram of LO extract (upper) and type III water as blank (lower).

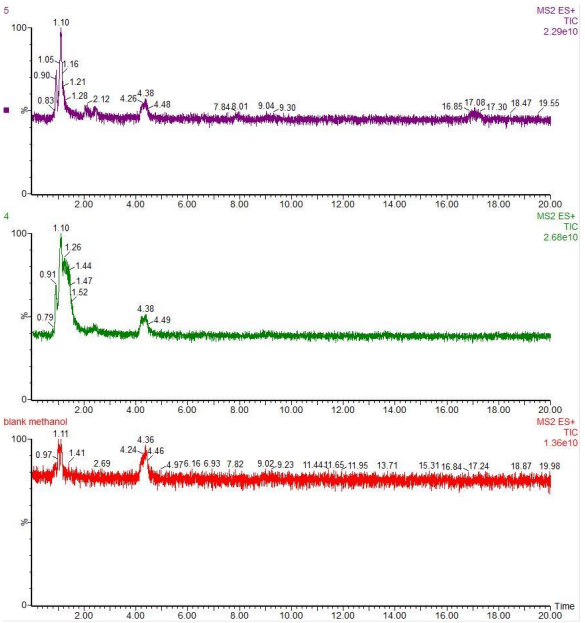


Figure 5. Chromatogram of MP extract (upper), PN extract (middle), and methanol as blank (lower)

Table 2. LC-MS result for LO

MW	Compound Class	Possible Compound (s)	MW	Compound Class	Possible Compound (s)
164	Phenol	Eugenol	328	Alkaloid	(-)-Litcubine, (-)-8-O-Methyloblongine
200	Fatty acid	Lauric Acid	337	Alkaloid	atheroline
220	Sesquiterpene	Humulene oxide	339	Alkaloid	Dicentrine, Litebamine
256	Fatty acid	Pinocembrin, Pinocembrin Chalcone, Palmitic acid*	342	Alkaloid	Magnoflorine* Phyllocoumarin
282	Fatty acid	Oleic Acid	343	Diterpenes	N-Feruloyl-3-methoxytyramine* Glaucine
286	Flavonoid	Kaempferol	356	Lignan	Balanophonin B* Xanthoplanine
295	Alkaloid	Ushinsunine	371	Alkaloid	(+)-N-(Methoxycarbonyl)-N-norboldine
296	Diterpene	Trans-phytol	386	Lignans	(+)-Eudesmin
314	Alkaloid	(-)-Litcubinine (-)-Magnocurarine (-)-Oblongine	433	Flavonoid	pelargonidin 3-glugoside, pelargonidin 5-glucoside-
326	Lignan	Dehydrodieugenol* dehydrodiisoeugenol			

Out of the 19 compounds in the MP library, 1 compound matched with the LC-MS peak at mw 256. The LC-MS for PN, conversely, did not show any corresponding peaks with the 16 compounds in the PN compound library.

3.2 Discussion

Phytochemical Screening and Assay

For phytochemical screening, the crude extracts were diluted to 2000 ppm as this concentration allows for the observation of visible colour change in extracts, while still concentrated enough to react with reagents. The phytochemical screening for flavonoids turns yellow in positive result due to the reaction of flavonoid with NaOH which produces acetophenone which is yellow in colour (Fransina, 2019). The phytochemical screening of flavonoids shows a presence of flavonoids in

LO and MP extracts. This result is consistent with the total flavonoid content assay, which reveals 0.993 ± 0.401 QE mg/g and 14 ± 2.663 QE mg/g total flavonoid content in LO and MP respectively.

Phenolics and tannin screening was conducted through a ferric chloride test. This test works as $FeCl_3$ reacts with phenol groups at a neutral pH, resulting in compounds with different colours depending on the type of phenol group present. A positive reaction could be indicated by either the colour red, green, blue, or purple (Boufellous et al., 2017). However, some phenols would not give this positive colorimetric result. Therefore, a negative colorimetric test result should not be directly interpreted as the absence of phenol without supporting information (Dhingra & Ahluwalia, 2004). In this case, a negative $FeCl_3$ result was seen in PN extract. The quantification analysis, however, shows that the extract does contain phenols at 48.654 ± 1.239 GAE mg/g. Therefore, the seemingly negative result in phytochemical screening of PN extract was proven to be wrong. On the other hand, both LO and MP extracts show a presence of tannin or phenolic compounds as the colour of their solution darkened from yellow and green respectively to brown, which is the result of the formation of red colour. The total phenolic content assay of LO and MP supports this screening result, where LO contains 33.559 ± 0.783 GAE mg/g and MP 78.572 ± 1.565 GAE mg/g.

A positive mayers' test for alkaloid is indicated by the presence of greenish or creamy colour precipitation (Owusu, 2021). The phytochemical screening of alkaloids in all extracts showed no formation of white precipitate, but LO extract did change in colour to light green, which was taken as a positive result. The rest of the extracts show no visible colour change upon the addition of Mayer's reagent. However, this negative screening result was proven to be due to the limit of detection of human eyes for colour change, as the result of the total alkaloid content assay shows presence of small amounts of alkaloid in both extracts. The result of the total alkaloid content assay found in PN, MP, and LO ethanolic extract were the following : 25.356 ± 11.336 AE mg/g, 19.045 ± 3.676 AE mg/g, and 7.880 ± 1.401 AE mg/g extract respectively.

LC-MS

The identification of compounds through LC-MS could be done through either comparing the MS data with a standard, or to a compound library. Since there is very limited data regarding the probable phytochemical constituents of each species, obtaining a sample standard would be ineffective. Therefore, the analysis was conducted based on the researcher's compiled compound library.

The chromatogram and mass spectrum of each probable compound was compared with the blank of each extract. For LO, 30 compounds with 20 different molecular weights in the library have at least one matching peak with the LC-MS result. Since the compound library of LO and MP were generated from their genus while PN was taken from its neighbouring species and not their species

specifically, it is impossible to identify the compounds only through the presence of one or two peaks in the MS. Therefore, an analysis of the fragmentation peaks was conducted. Compounds with the same molecular weights were narrowed down by analysis of their fragmentation patterns. Only one molecule was found to match 4 fragments of its molecule, which was eugenol in LO extract.

For MP, only one peak at mw 256 corresponded with the compound library. The possible compound based on the library is hexadecanoic acid. The peaks in PN extract LC-MS result did not match with the compound library gathered from *Pogonatum inflexum* species. To determine if other suspected compounds are present in all three extracts and elucidate compounds, further analysis should be done using standards or through nuclear magnetic resonance analysis (NMR) (Tomou, 2020).

CHAPTER 4: CONCLUSION AND RECOMMENDATION

Conclusion

The phytochemical content of MP, PN, and LO ethanolic extract were investigated. All extracts do not contain saponin. The major group of secondary metabolites contained in LO are phenolics, and the extract also contains small amounts of alkaloid and flavonoids. Based on the LC-MS result, one of the phenolics that is known to be contained in the plant is eugenol. MP contains the highest concentration of phenolics compared to the other extracts. It also contains small amounts of alkaloids and flavonoids. PN contains the highest concentration of alkaloids compared to the other extracts, however their major phytochemical constituents are phenolics. This extract does not contain flavonoids.

Recommendations

The lack of compound library for each species made it impossible to characterise peaks in the LC-MS result. Therefore, further studies could be done to elucidate these compounds through NMR analysis.

CHAPTER 5: SELF REFLECTION

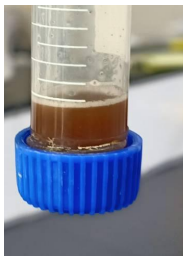
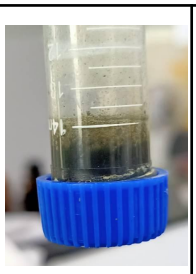
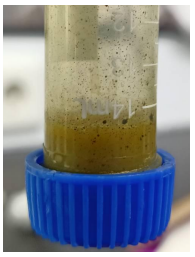
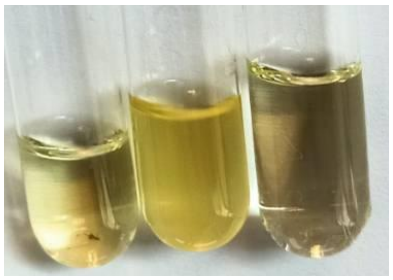
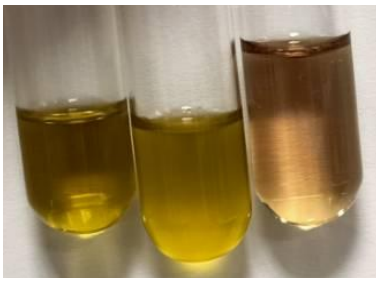
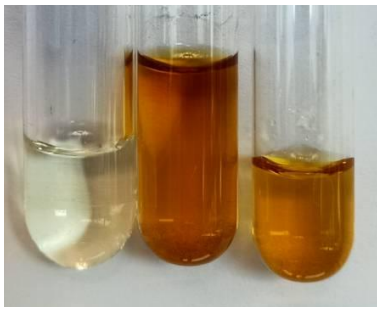
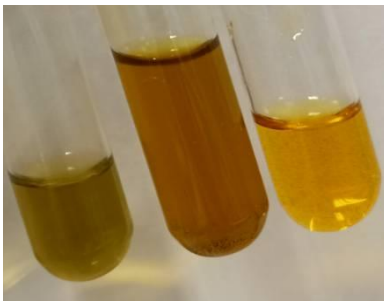
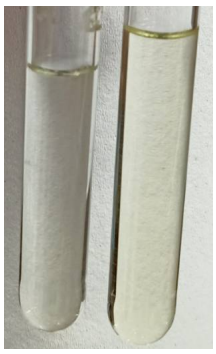
During my internship, I have gained several hard skills in the lab, and also used equipment I otherwise would not get a chance to use. Other than that, this internship period has helped me to understand how research works in real life : how I could plan everything as well as I can, but in the end not everything will always go my way and I have to adapt. The first problem I ran into was limited time for the experiment. Through this short amount of time, I learnt to manage my time and activities well so all lab activities could be completed.

The second problem I ran into was expecting my results to be good the first time around. I realised very quickly that what may look simple on paper might not be as simple in real life. I ended up having to redo my phytochemical assays as the standard curves were not up to the linear standard that was acceptable. During this time, I learnt to be patient with myself and not rush things.

The third expectation I had was that I would be doing the phytochemical screening alone. It turns out that along the way, I was helped by so many people ranging from my friends who were in the same project to upper cohort alumni and faculty didn't have to help me but did anyway. To Ko Gio and Sir Fandi who helped me in doing LC-MS, I am so grateful. I am also grateful for Vivian, Jason, Crishella, and Sun Joshua who helped me do my project repetitions. Lastly, I would also express my gratitude to my field supervisor Miss Pietra, and also Ci Erika as her research assistant who were always there to help me whenever I ran into a problem.

APPENDICES

Appendix 1. Phytochemical Screening of Extracts

	LO		PN		MP	
Saponin						
	0 minute	10 minutes + HCl	0 minute	10 minutes + HCl	0 minute	10 minutes + HCl
Flavonoid						
	Left : Sample Middle : Sample + NaOH Right : Sample + NaOH + HCl		Left : Sample Middle : Sample + NaOH Right : Sample + NaOH + HCl		Left : Sample Middle : Sample + NaOH Right : Sample + NaOH + HCl	
Phenolic / Tannin						
	Left : Sample Middle : sample + $FeCl_3$ Right : $FeCl_3$		Left : Sample Middle : sample + $FeCl_3$ Right : $FeCl_3$		Left : Sample Middle : sample + $FeCl_3$ Right : $FeCl_3$	
Alkaloid						
	Left : Sample Right : Sample + sample reagent		Left : Sample Right : Sample + sample reagent		Left : Sample Right : Sample + sample reagent	

Appendix 2. Compound Library for the genus *Litsea*

No	Compound Class	Compound Name	**MW**	No	Compound Class	Compound name	**MW**
1	alkaloid	Isoboldine	**146**	37	Flavonoid	Pinocembrin	**256**
2	fatty acids	2,6-Dimethyl-6-hydroxy-2E,4E-hepta-2,4-dienal	**154**	38	Flavonoid	Pinocembrin chalcone	**256**
3	lignans	Eugenol	**164**	39	fatty acids	Palmitic acid	**256**
4	fatty acids	Litseacubebic acid	**170**	40	Flavonoid	Apigenin	**270**
5	fatty acids	Cis-Dec-4-enoic acid	**170**	41	Flavonoid	Pinostorbin	**270**
6	fatty acids	Capric acid	**172**	42	fatty acids	Linoleic acid	**280**
7	fatty acids	Cis-Dodec-4-enoic acid (Linderic acid)	**198**	43	fatty acids	Oleic acid	**282**
8	fatty acids	Lauric acid	**200**	44	fatty acids	Stearic acid	**284**
9	fatty acids	6,7-Dihydroxy-3,7-dimethyl-oct-2-en oic acid	**202**	45	fatty acids	Ethyl palmitate	**284**
10	Sesquiterpene	Aromadendrene	**204**	46	alkaloid	Coclaurine	**285**
11	Sesquiterpene	α-Cadinene	**204**	47	alkaloid	Norjuziphine	**285**
12	Sesquiterpene	β-Cadinene	**204**	48	Flavonoid	Kaempferol	**286**
13	Sesquiterpene	γ-Cadinene	**204**	49	Flavonoid	Catechin	**290**
14	Sesquiterpene	δ-Cadinene	**204**	50	Flavonoid	Epicatechin	**290**
15	Sesquiterpene	β-Elemene	**204**	51	alkaloid	Ushinsunine	**295**
16	Sesquiterpene	γ-Elemene	**204**	52	Diterpenes	Trans-Phytol	**296**
17	Sesquiterpene	Germacrene	**204**	53	alkaloid	Glaziovine	**297**
18	Sesquiterpene	α-Humulene	**204**	54	alkaloid	Juziphine	**299**
19	Sesquiterpene	Ledene	**204**	55	Flavonoid	quercetin	**302**
20	Sesquiterpene	α-Amorphene	**204**	56	alkaloid	Laetine	**311**
21	Sesquiterpene	β-Caryophyllene	**204**	57	alkaloid	Actinodaphnine	**311**
22	Sesquiterpene	α-Copaene	**204**	58	alkaloid	Litseferine	**311**
23	Sesquiterpene	Humulene oxide	**220**	59	fatty acids	Ethyl stearate	**312**
24	Sesquiterpene	α-Cadinol	**222**	60	alkaloid	Laurelliptine	**313**
25	Sesquiterpene	Chromolaevanedione	**222**	61	alkaloid	Laurolitsine	**313**
26	Sesquiterpene	Elemol	**222**	62	alkaloid	Laurotetanine	**313**
27	Sesquiterpene	α-Eudesmol	**222**	63	alkaloid	Lindcarpine	**313**
28	Sesquiterpene	β-Eudesmol	**222**	64	alkaloid	Norisoboldine	**313**
29	Sesquiterpene	γ-Eudesmol	**222**	65	alkaloid	Laetanine	**313**
30	Sesquiterpene	Bulnesol	**222**	66	alkaloid	(-)-Litcubinine	**314**
31	fatty acids	Cis-Tetradec-4-enoic acid	**226**	67	alkaloid	(-)-Magnocurarine	**314**
32	lignans	Biseugenol A	**228**	68	alkaloid	(-)-Oblongine	**314**
33	fatty acids	Myristic acid	**228**	69	Diterpenes	Cubelin	**316**
34	Sesquiterpene	Aphanamol II	**236**	70	alkaloid	Cassameridine	**319**

35	lignans	Biseugenol B	**242**	71	alkaloid	Isodomesticine	**325**
36	fatty acids	Hexadecenoic acid	**254**	72	alkaloid	Cassythicine	**325**
73	alkaloid	Nordicentrine	**325**	100	alkaloid	Xanthoplanine	**356**
74	alkaloid	Phanostenine	**325**	101	lignans	Balanophonin B	**356**
75	lignans	Dehydrodieugenol	**326**	102	fatty acids	Lignoceric acid	**368**
76	lignans	Dehydrodiisougenol	**326**	103	alkaloid	(+)-N-(Methoxycarbonyl)-N-norboldine	**371**
77	alkaloid	Boldine	**327**	104	lignans	(+)-Eudesmin	**386**
78	alkaloid	Corytuberine	**327**	105	lignans	(+)-medioresinol	**388**
79	alkaloid	N-Methyllindcarpine	**327**	106	steroids	B-sitostenone	**412**
80	alkaloid	Norcorydine	**327**	107	steroids	Stigmasterol	**412**
81	alkaloid	Norisocorydine	**327**	108	lignans	(+)-Epiexcelsin	**414**
82	alkaloid	Pallidine	**327**	109	steroids	β-Sitosterol	**414**
83	alkaloid	(-)-Litcubine	**328**	110	lignans	Syringaresinol	**418**
84	alkaloid	(-)-8-O-Methyloblongine	**328**	111	Flavonoid	Afzelin	**432**
85	alkaloid	Reticuline	**329**	112	lignans	Grandisin	**432**
86	alkaloid	Dicentrinone	**335**	113	Flavonoid	Pelargonidin 3-glugoside	**433**
87	alkaloid	Atheroline	**337**	114	Flavonoid	Pelargonidin 5-glucoside	**433**
88	alkaloid	Dicentrine	**339**	115	Flavonoid	Kaempferol 3-O-b-d-galactopyranoside (trifolin)	**448**
89	alkaloid	Litebamine	**339**	116	Flavonoid	Quercitrin	**448**
90	alkaloid	Corydine	**341**	117	Flavonoid	Kaempferol 7-glucoside	**448**
91	alkaloid	Litseglutine B	**341**	118	Flavonoid	Astragalin	**448**
92	alkaloid	N-Methyllaurotetanine	**341**	119	Flavonoid	Isoquercetin	**464**
93	alkaloid	Predicentrine	**341**	120	Flavonoid	Quercetin 3-O-b-d-galactopyranoside	**464**
94	alkaloid	Sebiferine	**341**	121	lignans	Glochidioboside	**522**
95	alkaloid	Isocorydine	**341**	122	Steroids	Daucosterol	**576**
96	Flavonoid	phyllocoumarin	**342**	123	lignans	9,9'-O-di-(E)-feruloyl-(+)-secoisolariciresinol	**580**
97	alkaloid	Magnoflorine	**342**	124	Flavonoid	Naringin	**583**
98	amides	N-Feruloyl-3-methoxytyramine	**343**	125	steroids	Daucosterol	**594**
99	alkaloid	Glaucine	**355**	126	Flavonoid	Tiliroside	**714**

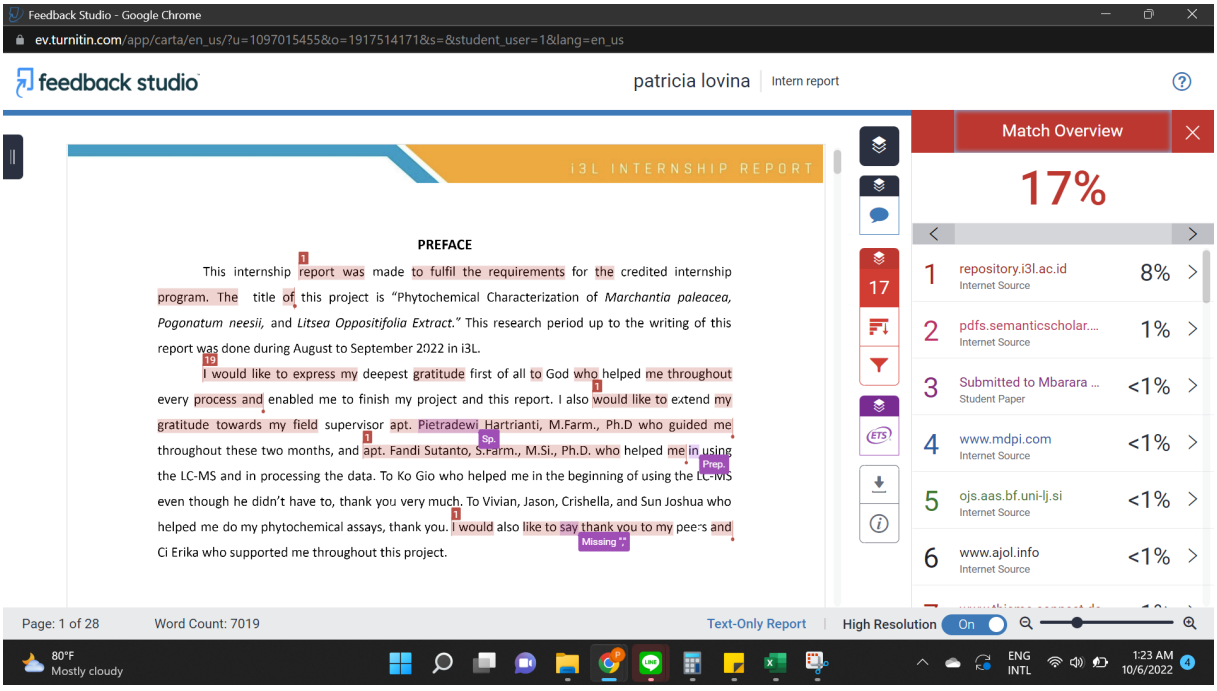
Appendix 3. Compound Library for the genus *Marchantia*

No	Compound Class	Compound Name	MW	No	Compound Class	Compound Name	MW
1	isoprenoid	Cuparene	**202**	11	Flavonoids	Apigenin	**270**
2	isoprenoid	Hujopsene	**204**	12	fatty acid	Octadecanoic acid	**284**
3	isoprenoid	Acoradiene	**204**	13	Flavonoids	Luteolin	**286**
4	isoprenoid	ß-chamigrene	**204**	14	Flavonoids	Quercetin	**302**
5	isoprenoid	ß - himachalene	**204**	15	sterol	Stigmasterol	**412**
6	isoprenoid	γ-cuprenene	**204**	16	sterol	22, 23-dihydrostigmasterol	**414**

7	isoprenoid	α - chamigren-9-one	**204**	17	phenol	Plagiochin E	**424.**
8	Flavonoids	5-hydroxyl-7-methoxyl-2-methylchromone	**206**	18	phenol	Marchantin A	**440**
9	hydroxybenzaldehyde	p-hydroxybenzaldeyde	**228**	19	Flavonoids	Apigenin-7-O-b-D-glucuronide	**446**
10	fatty acid	n-hexadecanoic acid	**256**				

Appendix 4. Compound Library for the genus *Pogonatum*

no	Compound Class	Compound Name	MW	no	Compound Class	Compound Name	MW
1	phenolic aldehyde	Protocatechuic aldehyde	**138**	9	flavonoid	Luteolin	**286**
2	phenolic aldehyde	2-hydroxy-5-(2-hydroxy-4-methoxybenzyl)-4-methoxybenzaldehyde	**152**	10	flavonoid	5,2'-dihydroxy-6,7-methylenedioxy-flavanone	**300**
3	phenolic aldehyde	4-hydroxy-3-methoxy-benzaldehyde	**153**	11	flavonoid	Kaempferide	**300**
4	flavonoid	Tricine	**179**	12	flavonoid	Quercetin	**302**
5	flavonoid	Apigenin	**270**	13	flavonoid	5,2',3'-trihydroxy-6,7-methylenedioxyflavanone	**316**
6	flavonoid	Baicalein	**270**	14	flavonoid	3,5,4'-trihydroxy-7,3'-dimethoxyflavone	**330**
7	flavonoid	Naringenin	**272**	15	flavonoid	3,5,3'-trihydroxy-7,4'-dimethoxyflavanone	**330**
8	flavonoid	Kaempferol	**286**	16	flavonoid	Irisflorentin	**386**



Appendix 5. Turnitin similarity result

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