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# EFFECT OF ETHANOLIC SOLVENT POLARITY ON THE PHYSIOCHEMICAL PROPERTIES OF *Graptophyllum Pictum. L*

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**Abstract:**

This study aims to compare the total phenolic content (TPC) and total flavonoid content (TFC) of *Graptophyllum pictum (L.) Griff* or *G. pictum (L)*. The leaves of *G. pictum (L)* were dried under 50ºC in a drying oven for 24 hours and then were ground by using a grinder until a uniform fine powder was obtained. Sample extraction was done by maceration technique for 7 days with three different ethanolic percentages, 50%, 70%, and 80% to be then concentrated with a rotary evaporator under 45 ºC. Qualitative analysis of *G. pictum (L)* extracts was done by two tests, the Ferric Chloride test for phenolics and the Shinoda test for flavonoids. Ethanol was used as the negative control while gallic acid as the positive control for TPC and quercetin for TFC. *G. pictum (L)* extracts were found to have the presence of both phenolics and the flavonoids compound by the formation of bluish-black colour for the ferric chloride test and the formation of pink scarlet colour for the Shinoda test. Standard calibration curves of gallic acid and quercetin were prepared. The quantitative analysis was carried out with a phytochemical screening of *G. pictum (L)* extracts using a standard procedure revealing the absorbance value of TPC and TFC. Folin Ciocalteu's method was done to identify the absorbance of TPC while the aluminum chloride method was done for determining the absorbance of TFC through a UV-VIS spectrophotometer. 50% ethanolic solvent yielded the highest TPC and TFC (84.24 ± 0.09 mg GAE/g and 19.824 ± 0.08 mg QE/g respectively). Statistical analysis was done for all results obtained through ANOVA. There is no significant difference in absorbance TPC and TFC value between the ethanolic percentages. Despite that, in conclusion, 50% ethanolic solvent came out the best in extracting the phytochemicals. *G. pictum (L)* has the potential to be a useful resource in the development of a drug for the treatment of PCOS.

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**Keywords:**

*Graptohyllum Pictum. L*, Phenolic, Flavonoid

## Introduction

Infertility is defined as the inability to conceive and produce a child naturally without any medical intervention and about 8-10% of couples experience infertility issues, and the factors that can influence conception in couples include weight, food, smoking, other substance misuses, environmental contaminants, infections, medical conditions, medications, and family medical history (Patel & Modi, 2018). The most common cause of infertility in women is a condition known as polycystic ovarian syndrome (PCOS), which is exacerbated by obesity, and the development of uterine fibroids and endometriosis (Abdullah et al., 2016). Menstrual disorders which are defined as disorders of cycle length and flow accounted for 62.6 % of infertility in women (Masoumi et al., 2015). Polycystic ovaries are associated with a high likelihood of infertility and women are more likely to have trouble getting pregnant and will need treatment to boost back the chances of getting pregnant.

Herbal or alternative treatments have been proven to balance female hormones by reducing male hormones, restoring the oestrous cycle, and reducing insulin resistance. Herbal medicines also have positive benefits such as enhancing lipid metabolism, containing anti-inflammation, and anti-oxidative stress, and suppressing autophagy, and/or apoptosis. (Kwon et al., 2020). Throughout the years, humans have been using various types of therapeutic herbs and plants to cure and treat diseases. *Graptophyllum pictum (L.) Griff* or *G. pictum (L)*, is a member of the Acanthaceae family and it has also been empirically used as a traditional antidiabetic drug in Indonesia (Nugroho, 2020). *G. pictum (L)*, a traditional Indonesian medicinal herb that has anti-inflammatory characteristics commonly used due to the secondary metabolic content such as flavonoids, tannins, non-toxic alkaloids, steroids, saponins, and glycosides (Juniarti et al., 2020). Even local folks use the tea made from this plant to cure the imbalanced menstrual cycle and control the blood flow after a mother gives birth.

This proves that herbal medications have the potential to be valuable resources in the development of successful PCOS therapy. Having to say that this research aims to find the effect of ethanolic solvent percentages of 50%, 70%, and 80% on the crude extract of *G. pictum (L)* along with investigating the total phenolic content (TPC) and total flavonoid content (TFC). Therefore, the methods that were performed in this study included the extraction of *G. pictum (L)* leaves and the qualitative and quantitative analysis of phytochemical screening in the leaves by absorbance measurement using a UV-VIS spectrophotometer. This is to identify the amount of content that could have medicinal properties for PCOS treatment. *G. pictum (L)* has the potential to be a valuable resource in the development of PCOS therapeutics, and it is hoped that with this, the discovery of medications for treating patients with PCOS can be established.

## Literature Review

### *Graptophyllum pictum. L or G. pictum (L)*

*Graptophyllum pictum (L)* or *G. pictum (L)* are best known as *"Daun Ungu"* (violet leave) in Indonesia or *"Puding Belacan"* in Malaysia is a traditional herbal plant that is frequently grown as a shrub, consisting of two varieties which are one with variegated leaves, known as

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'white adulsa,' and another with dark leaves, known as 'black adulsa' (Singh, 2015). The Acanthaceae family's *G. pictum (L)* has been empirically utilized as a traditional antidiabetic medicine in Indonesia and the leaves have been used by the community to treat a variety of ailments, including haemorrhoids, ulcers, ear and stomach ulcers, and easing women's menstrual cycles (Nugroho, 2020). In Negeri Sembilan, the tender leaves of *G. pictum (L)* were usually used in the traditional cuisine as *"Rendang"* (Beef or Chicken Spicy Stew) or *"Gulai Masak Lemak Daging Salai* (Smoke Beef in a Coconut Gravy) because of the plant medicinal properties and the plant is widely available in Negeri Sembilan's state. Besides that, the *G. pictum (L)* also being boiled with water and serve as a herbal tea. Figure 1 shows the picture of *G. pictum (L)* leaves.

**Figure 1: *G. pictum (L)* Leaves**

*G. pictum* leaves contain flavonoids, which can bind reactive oxygen species, as well as alkaloids, which have the potential to treat a variety of blood glucose-related disorders (Bharti, et al., 2018; Nugroho, 2020). The skin and leaves of *G. pictum (L)* are usually slimy and have an unpleasant odour. It also has an obtuse angle, is pole-shaped, and is segmented, and the morphology of the flowers is held in a short terminal thyrse like bracts and bracteoles that are lanceolate around 2–3 mm long (Flora, 2019). *G. pictum (L)* has erect stems and can only reach 3 meters in height. The leaves are usually 8–20 cm long and 3–13 cm wide, and the upper surface is glossy purple. It can be found from the lowlands to the mountains at an altitude of 1,250 meters above sea level (Abidin & Jember, 2020). Modern pharmacological investigations have examined *G. pictum (L)* for anti-haemorrhoid, anti-microbial, anti-inflammation, anti-analgesic, and wound healing properties, which can be explained by the presence of numerous substances such as flavonoids, steroids, terpenoids, and anthocyanins (Makkiyah et al., 2021). Although *G. pictum (L)* has proved to have antioxidant properties, further research is still needed in order to obtain a more accurate extract composition and its effectiveness on chronic diseases. So, it is crucial to conduct extensive research into the phytochemical screenings for *G. pictum (L)*. A study conducted by Jiangseubchatveera and Pyne (2017) has proved the presence of the phytochemical screening along with the results of the amount of total phenolic and flavonoid contents of different types of solvent in the *G. pictum (L)*. The study has shown that

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the highest value of total phenolic content that was expressed in the term of milligrams of Gallic Acid Equivalents (mg GAE/g) was by ethyl acetate fraction which was 102.57±0.19 mg GAE/g, while the hexane fraction brought up the lowest value of total phenolic content, 11.69±0.09 mg GAE/g whereas, for the total flavonoid content, that was expressed in the term of milligrams Quercetin Equivalents (mg QE/g), hexane fraction showed the highest value of flavonoid content which was 28.21±0.04 mg QE/ g, while the aqueous fraction had the lowest total flavonoid content which was 2.02±0.02 mg QE/ g (Jiangseubchatveera & Pyne, 2017). However, though the previous study had the same concept for the phytochemical screening of total phenolic contents and total flavonoid contents of the *G. pictum (L)* extract, this study mainly focuses on comparing the effect of different ethanolic percentages (50%, 70%, and 80%) on the ability of the solvent to extract the phytochemical content in *G. pictum (L),* while the previous study focused on the effect of different types of solvent on the *G. pictum (L)* extract. Ethanol was chosen as a solvent for this extraction method because ethanol was proven to be efficient, easy to use, and could extract the high total amount of phenolics and flavonoid content in plant extract (Hikmawanti et al., 2021).

***Polycystic Ovary Syndrome (PCOS)***

Polycystic ovary syndrome (PCOS) is a diverse condition primarily affecting women due to a female endocrine reproductive disorder like a persistent hormonal disbalance that leads to complications such as numerous cysts and an irregular menstrual cycle, which eventually can lead to infertility among women (Ajmal et al., 2019). Polycystic ovarian syndrome (PCOS) is also a complicated illness marked by high testosterone levels, irregular menstrual cycles, and/or tiny cysts on one or both ovaries (Ndefo, Eaton & Green, 2013). Primary abnormalities in the hypothalamic–pituitary axis, insulin secretion and action, and ovarian function are all involved in the pathophysiology of PCOS (Franks & Hardy, 2020). Anovulation in polycystic ovarian syndrome (PCOS) can be characterized by the stoppage of antral follicles, which is a phenotype that can be saved by raising the concentrations of the circulation levels of follicle-stimulating hormone (FSH). Recent studies found that the cause of anovulation has been linked to abnormalities in gonadotropin regulation, secretion, and action, as well as intraovarian variables (Franks & Hardy, 2020).

According to the PCOS developmental theory, PCOS is caused by the female foetus being exposed to high levels of androgen during pregnancy, as well as the effects on steroidogenesis, insulin signalling, pancreatic b-cell function, hypothalamic–pituitary structure, neuroendocrine secretory patterns, and epigenetic alterations (Witchel et al., 2019). However, women who suffer from PCOS should make lifestyle improvements first before proceeding with fertility treatment. Letrozole is considered the first-line ovulation induction drug and can be replaced with clomiphene if letrozole is not accessible. In PCOS, ovulation induction can be difficult, especially when first-line agents fail to induce ovulation or when second-line agents are needed (Mascarenhas & Balen, 2020). Those first and second-line agents are the medicines that can treat PCOS. However, these medicines are synthetic drugs that have side effects on women, such as an increased risk of developing ovarian cancer and uterine cancer. This led to the need for the research and development of drugs made from natural sources, which were less expensive as it was made from a native plant and lowered the risk of developing cancer. According to findings by Kwon et al. (2020), herbal medicines can also be a treatment for PCOS because herbal medicines can reduce the level of nerve growth factor in PCOS, regulate apoptosis and/or autophagy in PCOS, reduce oxidative stress in PCOS, and inhibit the

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inflammatory conditions of PCOS. Thus, this proves that herbal medicines have the potential to be valuable resources in the development of successful PCOS therapeutics.

## Methodology

The study was done using the experimental approach. The study started when the sample was collected and prepared for an extraction process. The extraction process was done when the sample was soaked with 50%, 70 and 80% ethanolic solvent before it was concentrated using the rotary evaporator. All the crude extract was tested using qualitative analysis via Ferric Chloride and Shinoda test to ensure the existence of phenolic and flavonoids in the extract. After the qualitative analysis result was obtained, the crude extract was analysed further using Folin Ciocalteu and Aluminium Chloride reagent to estimate the sample's total phenolic and total flavonoid content via UV-VIS spectrophotometer. Finally, the statistical analysis was done using the ANOVA to test the significant difference between the crude extract. Figure 2 shows the flow chart for the method used in this study.

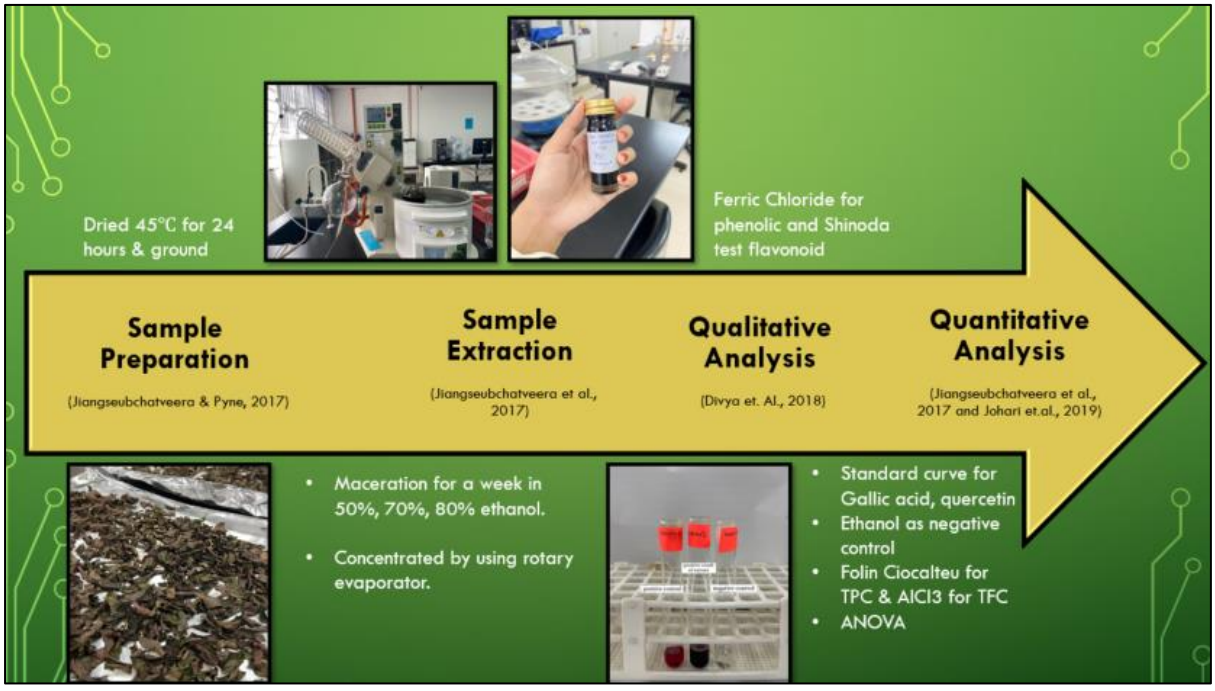


**Figure 2:Flow Chart for the Methodology**

### *Sample Preparation*

About two kilograms of *G. pictum (L)* leaves were gathered from the same location in Kuala Pilah district as the sample for this study. The sample was collected in October and November 2022. The sample was washed down with running tap water in the laboratory to remove all dirt and foreign materials. Then, the leaves were uniformly cut before being fully dried in the drying oven at 45 °C for 24 hours. The dried leaves were then ground using a grinder and sieved until the samples became a fine, uniform powder. The powder was weighed and kept in a 4 °C chiller (Jiangseubchatveera & Pyne, 2017). 150 g of *G. pictum (L*) powder was equally divided into three groups. Then, each of the 50 g of powder groups was soaked in 500 mL of ethanol with three different concentrations, which were 50%, 70%, and 80%, respectively. The soaking process was done in three 800 mL beakers, and all the groups were left to soak for a week in an orbital shaker with 100 rpm at room temperature. After a week, the mixture of powdered samples and the solvent from each group were filtered by using a vacuum pump with Whatman

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No 1 filter paper. The filtrates obtained were then concentrated and extracted by using a rotary evaporator. The concentrated crude extracts of *G. pictum (*L) from three different groups were obtained. All the crude extracts were kept in airtight glass bottles in a 4℃ chiller for future tests (Jiangseubchatveera & Pyne, 2017).

***Qualitative Test for Phenolic and Flavonoid in G. pictum (l)***

The presence of total phenolic content in *G. pictum (L)* leaves was determined by conducting a ferric chloride test. The methodology used was done according to Divya et al. (2018), with some slight changes. 3–4 drops of $FeCl_3$ solution were added and then swirled into 2 mL of extract in a test tube. A change in colour in the test tube will indicate the presence or absence of phenols in the extract. A change in colour from blue-green to blueish-black indicates a positive result for the presence of the phenol, while a change in colour to yellow indicates a negative result, which means that the phenol compounds are absent.

The presence of flavonoid compounds in *G. pictum (L*) leaves was determined by conducting a Shinoda test by referring to a methodology done according to Divya et al. (2018) with some modifications. 2 mL of the extract was poured into a test tube with a few magnesium ribbon pieces. Then, a few drops of concentrated HCl were added to the same test tube containing the mixture of the extract and the magnesium ribbon pieces. The presence of the pink scarlet colour indicates a positive result for the flavonoid compounds present in the leaves, while the absence of the pink scarlet colour indicates a negative result, which means there are no flavonoid compounds present.

***Quantitative Test for Phenolic and Flavonoid G. pictum (L)***

A standard calibration curve was prepared by following a methodology developed by Shirazi, et al., (2014). Gallic acid was used as the positive control of the standard calibration curve, while ethanol acted as the negative control of the curve. Firstly, 1 g of gallic acid was dissolved in 100 mL of ethanol to get 1% of gallic acid (10 mg/mL) and termed a standard solution. Then, a standard gallic acid curve was plotted by preparing a serial dilution of 0.1, 0.5, 1.0, 2.5, and 5 mg/mL in ethanol from a standard solution of gallic acid. A calibration curve was obtained by measuring the absorbance using the UV-VIS spectrophotometer at 760 nm.

A methodology developed by Shirazi et al. (2014) was followed to obtain a standard calibration curve for quercetin. 1 g of quercetin was dissolved in 100 mL of ethanol to make up 1% of quercetin (10 mg/mL) and termed a standard solution. Then, a standard quercetin curve was plotted by preparing a serial dilution of 0.1, 0.5, 1.0, 2.5, and 5 mg/mL in ethanol from the standard solution earlier. Quercetin acted as the positive control while ethanol acted as a negative control. A calibration curve was obtained by measuring the absorbance using the UV-VIS spectrophotometer at 510 nm.

The total phenolic content of the *G. pictum (L)* leaf extract was estimated by using Folin Ciocalteu's reagent as described by Jiangseubchatveera & Pyne, (2017) with slight modification. Firstly, 0.5 mL of extract was mixed with 2.5 mL of Folin Ciocalteu's reagent in the test tube. After 3 minutes, the mixture was mixed subsequently with 2 mL of saturated $Na_2CO_3$. The mixture was then allowed to stand in the dark for 30 minutes at room temperature. The mixture absorbance was then measured at 760 nm by using the UV-VIS spectrophotometer. The gallic acid acts as the positive control while the ethanol solvent acts as a negative control. The results were expressed as milligrams of gallic acid per gram of extract

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(mg/GAE) per gram extract. The same steps were repeated for each extract with different solvent concentrations. All of the steps were performed in triplicate.

The total flavonoid content was measured spectrophotometrically by using the $AlCl_3$ reagent method as described by Jiangseubchatveera & Pyne (2017). Firstly, 1 mL (1 mg/mL) of the extract was mixed with 1 mL of 2% $AlCl_3$ in ethanol. Then, the mixture was incubated at room temperature for 10 minutes. The absorbance of the sample was measured by using UV-VIS spectrophotometer at 510 nm against the blank sample consisting of the solvent which is ethanol. The quercetin acts as the positive control while the ethanol solvent acts as the negative control. The content of flavonoids was expressed in terms of quercetin equivalent (mg QE) per gram extract. The same steps were repeated for each extract with different solvent concentrations. All procedures were done in triplicate.

For this study, the data results of the experiment involved which are TPC and TFC are all expressed as mean ± standard deviation of the triplicates value of each extract. The results were analyzed in Analysis of Variances (ANOVA) to compare the total phenolic and flavonoid content between all the crude extracts of *G. pictum (L)* from three different solvent concentrations which were 50%, 70%, and 80%.

## Result and Discussion

### *Qualitative Content of Total Phenolic (TPC) and Total Flavonoid (TPC) G. pictum (L)*

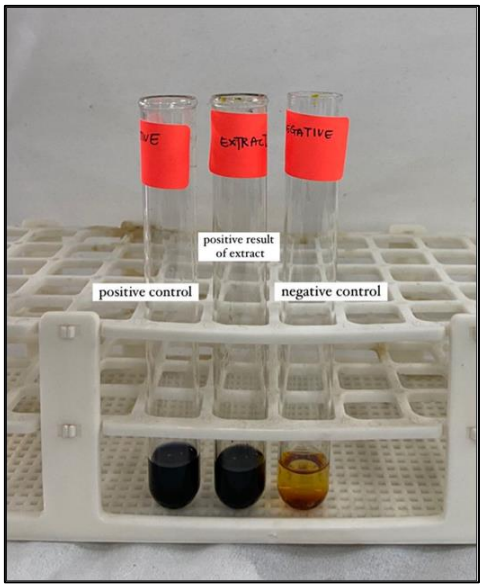


**Figure 3: Result for Ferric Chloride Test of *G. pictum (L)* Extract.**

Figure 3 shows the ferric chloride test for the solutions from the positive control (on the left), the *G. pictum (L)* extract (in the middle), and the negative control (on the right). This test was used to indicate the presence of phenol in the solution. A previous study stated that the formation of bluish-black colour indicated the presence of phenolic content and that this was due to the formation of a phenol-ion complex (Gonfa et al., 2020). Thus, the result above showed that *G. pictum (L)* extract does contain phenolic content.

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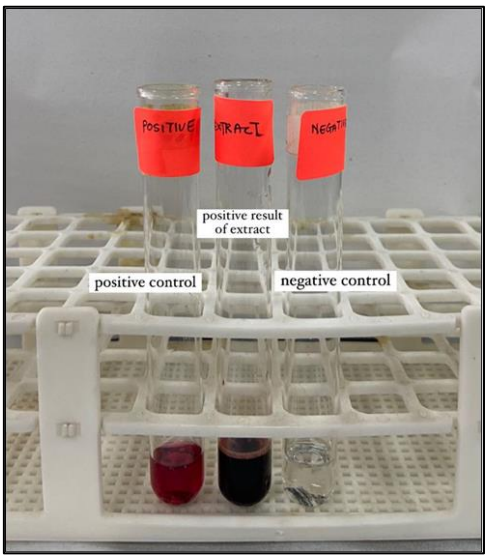


**Figure 4: Results for Shinoda Test of *G. pictum (L)***

Figure 4 shows the results of Shinoda for flavonoids. The results showed that the positive control (test tube on the left) along with *G. pictum (L)* extract (middle test tube) had a formation of pink-scarlet colour. A previous study done by Divya et al. (2018), stated that the presence of pink-scarlet colour indicates a positive result for flavonoid compounds, while the absence of pink scarlet colour indicates a negative result (test tube on the right). This Shinoda test is for the presence of flavones. If flavones are present in the test sample, the Shinoda reaction will reduce the flavonoids to anthocyanidins thus the formation of pink-scarlet occurs (Saptarini et al., 2016). As *G. pictum (L)* extract showed a formation of pink-scarlet colour after the test this concludes that there is the presence of flavonoid compounds.

***Quantitative Content of Total Phenolic (TPC) and Total Flavonoid (TFC)For G. pictum (L)***

**Table 1:Total Phenolic Content of *G. pictum (L)* Extracts from Three Ethanolic Concentrations. All Results Values Were Expressed As the Mean of Triplicate Determinations ± *SD***

| Ethanolic Concentration Percentage | TPC mg GAE/g |
|---|---|
| 50% | 84.24 ± 0.09 |
| 70% | 80.56 ± 0.03 |
| 80% | 83.32 ± 0.1 |

Table 1 shows the results of total phenolic content for all the extracts of *G. pictum (L)*. Total phenolic content was determined by measuring the absorbance using the spectrophotometric method using Folin Cicalteu's reagent. As mentioned by Jiangseubchatveera & Pyne (2017), in order to create a blue chromophore made of phosphotungstic-phosphomolybdenum complex, the Folin Ciocalteu was reduced by phenols in a basic condition. In this research, the total phenolic content of all three different ethanolic concentrations (50%, 70%, and 80%) of *G. pictum (L)* extracts was obtained and calculated from the plotted regression line of the gallic

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acid standard calibration curve (y = 0.2492x + 0.3155). The results were expressed in milligrams of gallic acid equivalent per gram (mg GAE/g). For TPC, there is no significant difference between all the concentrations of the extracts from the three solvent percentages obtained with p = 0.394% ($p \leq 0.05$).

From Table 1, the total phenolic content for extracts from different ethanolic concentration percentages was determined. 50% of ethanol yielded the highest amount of TPC (84.24±0.09 mg GAE/g), followed by 80% and 70% (83.32±0.1 mg GAE/g and 80.56±0.03 mg GAE/g respectively). Phenolic compounds that are commonly found in fruits, vegetables, and herbal plants are known to have high antioxidant activity (Mustafa et al., 2010). This is interesting to note that varying levels of phenolic content were found when the analysis of different solvent concentrations (50, 70, and 80%) was conducted. The solvent used in extraction and polarity affects how much of the phenolic compounds can be recovered. Most phenolic compounds that are responsible for the antioxidant capabilities are categorized as hydrophilic antioxidants (Joana, 2014). Thus, the difference in concentrations of the solvent, affects the polarity of the solvent, leading to an effect on the extraction yield of phytochemicals.

**Table 2:Total Flavonoid Content of *G. pictum (L)* Extracts from Three Ethanolic Concentrations. All Results Values were Expressed as Means of Triplicate Determinations ± SD.**

| Ethanolic Concentration Percentage | TFC MG QE/G |
|---|---|
| 50% | 19.824 ± 0.08 |
| 70% | 16.47 ± 0.25 |
| 80% | 17.295 ± 0.08 |

Table 2 shows the results of the total flavonoid content for all the extracts of *G. pictum (L)*. Total flavonoid content (TFC) was measured spectrophotometrically by using aluminium chloride, $AlCl_3$, as the reagent method. Flavonoids are a class of organic compounds with various phenolic structures and are well known for having beneficial health effects. The determination of the total flavonoid content of *G. pictum (L)* extracts was obtained from the regression equation of the Quercetin calibration curve (y = 0.2238x + 0.2312). The results were then expressed in milligrams of quercetin equivalent per gram (mg QE/g). The result between all the concentrations of the extracts from the three solvent percentages showed no significant difference with a p-value slightly higher than $p \leq 0.05$ ($p=0.056$).

Based on Table 2, 50% of the ethanol yielded the highest total flavonoid content, which is 19.824±0.08 mg QE/g, while 70% of the ethanol produced the lowest, which is 16.47±0.25 mg QE/g. This means that maximum flavonoids were extracted by 50% ethanol compared to 70% and 80%, and these results are convincing since the highest total phenolic content also resulted from 50% ethanol as solvent. Different concentrations of the solvent do play an important role in extracting the optimum level of flavonoid content contained in the samples. The previous study stated that polyphenols like flavonoids were most likely to be influenced by water concentration and heat. Chemicals that are soluble in water and/or organic solvent may be extracted more easily when water and organic solvent are combined, as this may explain why aqueous ethanolic solvent (50%) resulted in a higher TFC yield compared to 70% and 80%

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(Do et al., 2014). Phytochemicals such as flavonoids, which are polyphenols and have a high potential for antioxidant activity, also play a role in antibiotic activity because flavonoids make complexes with bacterial proteins, cell walls, and other ingredients that are accountable for biological action (Amir Hassan, 2020). Thus, using the best concentration of the solvent is vital to extracting the highest amount of TFC.

The method used to recover and isolate phytochemicals from *G. pictum (L)* was maceration extraction. The efficiency of extraction is usually affected by many properties and one of the properties is the polarity of the chosen solvent. For this study, three concentrations of ethanol (50%, 70%, and 80%) have been chosen as solvents to extract the phytochemicals contained in *G. pictum (L)*. Under the same extraction time and conditions, all three concentrations of ethanol extracts were tested, and the measurement of total phenolics and total flavonoid content was observed. From the results, the aqueous ethanolic extract (50%) obtained the highest yield for both TPC and TFC (84.24 ± 0.09 mg GAE/g and 19.824 ± 0.08 mg QE/g respectively) compared to 70% ethanolic extract (80.56 ± 0.03 mg GAE/g and 16.47 ± 0.25 mg QE/g respectively) and 80% ethanolic extract (83.32 ± 0.1 mg GAE/g and 17.295 ± 0.08 mg QE/g respectively). From the results, the amount of TPC and TFC was affected by the extraction yield. The extraction yield was affected by the polarity of the ethanol. A previous study stated that a higher concentration of water in the solvent improved the yield of the extraction (Do et al., 2014). According to Anwar and Przybylski (2012), aqueous solvents used for extraction were found to be more effective for the isolation and determination of phenolic compounds from different plant materials. The highest amount of TPC (428.3 ± 18.01 mg/g GAE) and TFC (16.3 ± 0.79 mg/g HE) was discovered in 70% ethanolic extract of *G. pictum (L)* (Kusumawati et al, 2022). The result differences from this study may be caused by differences in extraction technique, as the previous researcher used a microwave generator and then freeze-dried the sample. Heat and extraction conditions influenced the yield of TPC and TFC. However, another study conducted by Hikmawanti et al. (2021) proved that using 50% ethanol as an extraction solvent yielded the highest TPC and TFC value of *Sauropus androgynous* or "*katuk"* leaves with 42.18 mg GAE per gram and 11.18 mg QE per gram, respectively, compared to 70% and 96%. In comparison, this study showed that the highest value of TPC and TFC obtained also resulted from the aqueous ethanolic extract (50%), compared to 70% and 80% in the other samples. As the chemistry of the phytochemicals varies, different extraction concentration solvents may also have different effects and impacts on the extraction yield, solubility, and activity of the antioxidants in the phytochemicals. The amount of TPC and TFC obtained may be different depending on the chosen extracted plant. In some cases, there are plants that provide the highest number of phenolic contents by using a concentrated ethanolic solvent. However, for *G. pictum (L)*, an aqueous ethanolic solvent (50%) gave the optimum yield for TPC and TFC.

## Conclusion

The objective of this study had being achieved where the *G. pictum (L)* leaves had being process and the plant's physiochemical properties (total phenolic and total flavonoid content) was able to be obtained. Based upon the results of this study, a 50% ethanolic solvent concentration yielded the highest TPC and TFC of *G. pictum (L)*'s crude extract compared to 70% and 80%, though there are no significant differences between the solvent concentrations. *G. pictum (L)* has proven to have secondary metabolites that are beneficial for synthesizing new therapeutic treatments, mainly in PCOS.

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