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EXTRACTION OF HIBISCUS ROSA SINENSIS LEAVES USING ULTRASONIC WATER BATH IN DETERMINING THE PHENOLIC CONTENT.

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Abstract:

Hibiscus rosa-sinensis grows at many places around the globe. Hibiscus rosa-sinensis contains numerous classes of auxiliary metabolites, counting flavonoids, anthocyanins, terpenoids, steroids, polysaccharides, alkaloids, amino acids, lipids, and naphthalene group. Different kind of studies show that parts of *Hibiscus rosa-sinensis* plants possess antioxidant, antimicrobial, antidiabetic, antiulcer, hepatoprotective, antifertility, antigenotoxic and anti-inflammatory properties that help in treatment of many diseases. Therefore, *Hibiscus rosa-sinensis* plant has been identified as one of many medicinal plants that possesses various potential in therapeutic applications. Bioactive compound extraction on plants done by several methods like conventional method, Microwave Assisted Extraction (MAE) method etc. However, none of the study has been done on extraction of bioactive compounds extracted from Hibiscus rosa-sinensis leaves using Ultrasonic Assisted Extraction (UAE) method. Hence, there is essential to investigate on the benefits of the *Hibiscus rosa-sinensis* leaves for the future used by using UAE method for extraction. The objectives of this study are to extract bioactive compound extract from *Hibiscus rosa-sinensis* leaves by using ultrasonic water bath using different solvents like ethanol, methanol, ethyl acetate and acetone and to analyse phenolic profile from the extract. The solvent is chosen based on the polarity due to the presence of hydroxyl group in determining the phenolic compound. *Hibiscus rosa-sinensis* leaves is prepared by drying at 50°C and pestled before extraction by different solvent. After that, the sample is placed in ultrasonic water bath at different time (5 min, 15 min, 30 min, 45 min, 60 min) and then the solvent is removed by using rotary evaporator. The result shows the highest extraction yield by UAE method is methanol and

then followed by ethanol, acetone, and ethyl acetate. After extraction, UV-VIS spectrophotometer was used to determine the phenolic content in *Hibiscus rosa-sinensis* leaves. Even though methanol has the highest yield of extraction, it is not the best solvent to determine the phenolic content when comparing it with standard calibration curve of Gallic acid. However, as for ethyl acetate, it has the lowest extraction yield but able to determine the phenolic content. As a conclusion, the yield of extraction does not affect the determination of the phenolic content.

***Keywords*:**
Hibiscus rosa sinensis, Folin-Ciocalteu method, DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay , phenolic compounds, flavonoids.

Objectives:

- To extract bioactive compound extract from *Hibiscus rosa sinensis* leaves by using ultrasonic water bath using different solvents like ethanol, methanol, ethyl acetate and acetone.
- To analyze antioxidant and phenolic profile from the extract.

Methodology:

Figure 1 The process flow on the process of extraction by Ultrasonic Assisted Extraction (UAE) method.

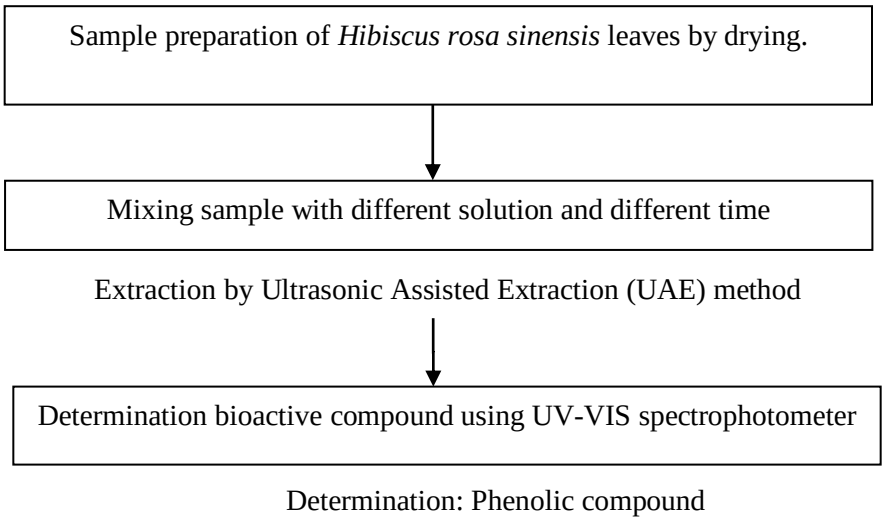

Figure 1 Process Flow of Project.

Results:

Table 1 Yield of extraction of Hibiscus rosa sinensis by using solvents (methanol, ethanol, acetone, and ethyl acetate)

Time (min)	Weight of sample after extraction (g)				Extraction Yield (%)			
	Methanol	Ethanol	Ethyl acetate	Acetone	Methanol	Ethanol	Ethyl acetate	Acetone
5	102.87	102.80	102.78	102.76	13	6	4	3
15	102.89	102.81	102.77	102.76	15	7	3	2
30	102.94	102.82	102.77	102.76	20	8	3	2
45	102.98	102.82	102.77	102.80	24	8	3	6
60	102.87	102.82	102.76	102.77	13	8	2	3
Average yield (%)					**17**	**7.4**	**3**	**3.2**

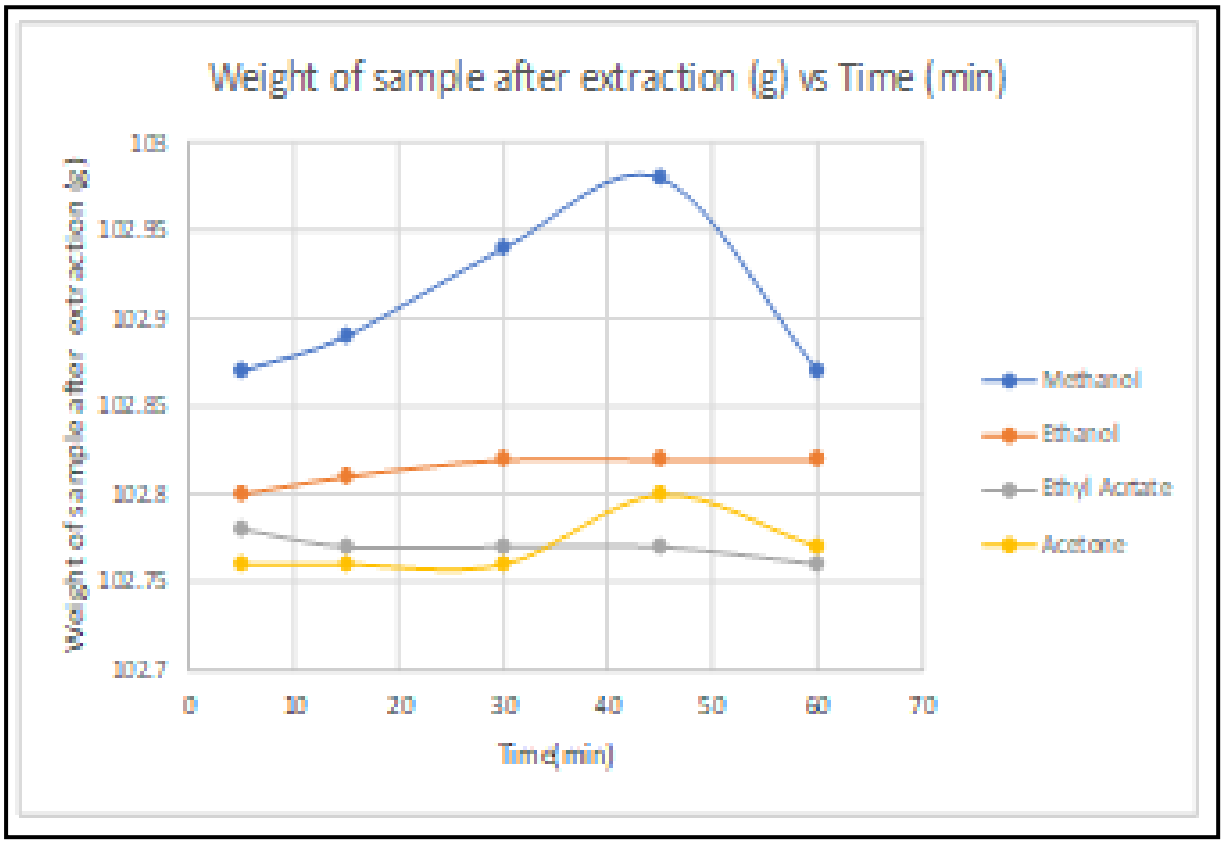

Figure 2 The weight of extraction over time

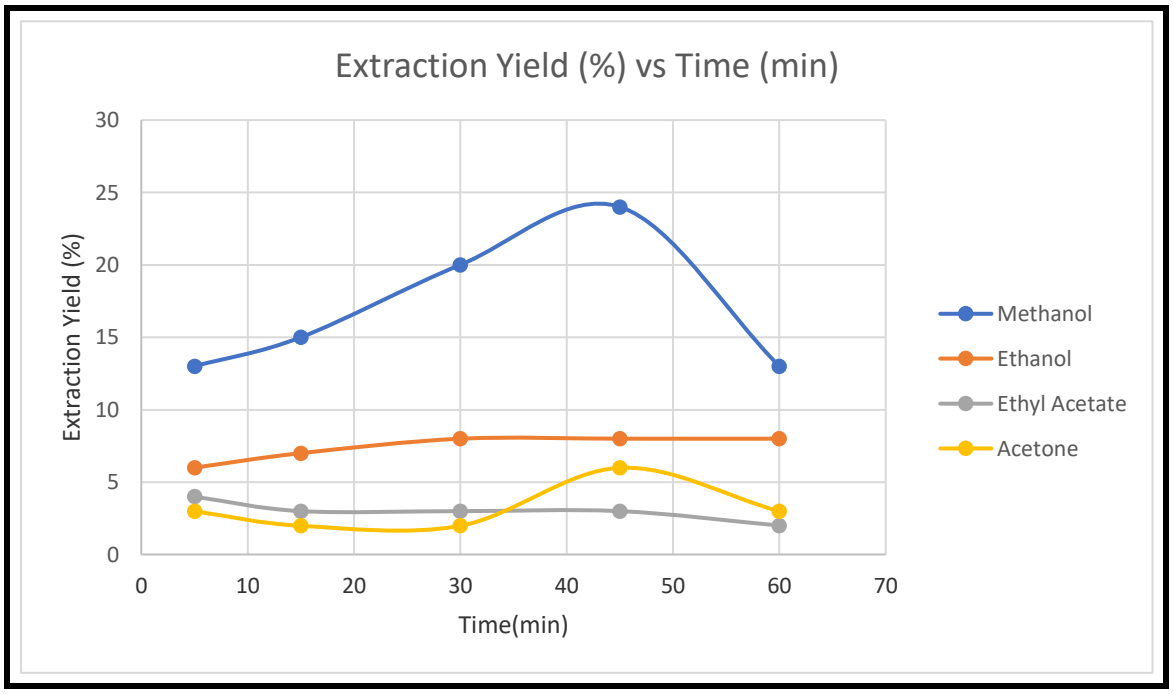

Figure 3 The Extraction yield over time

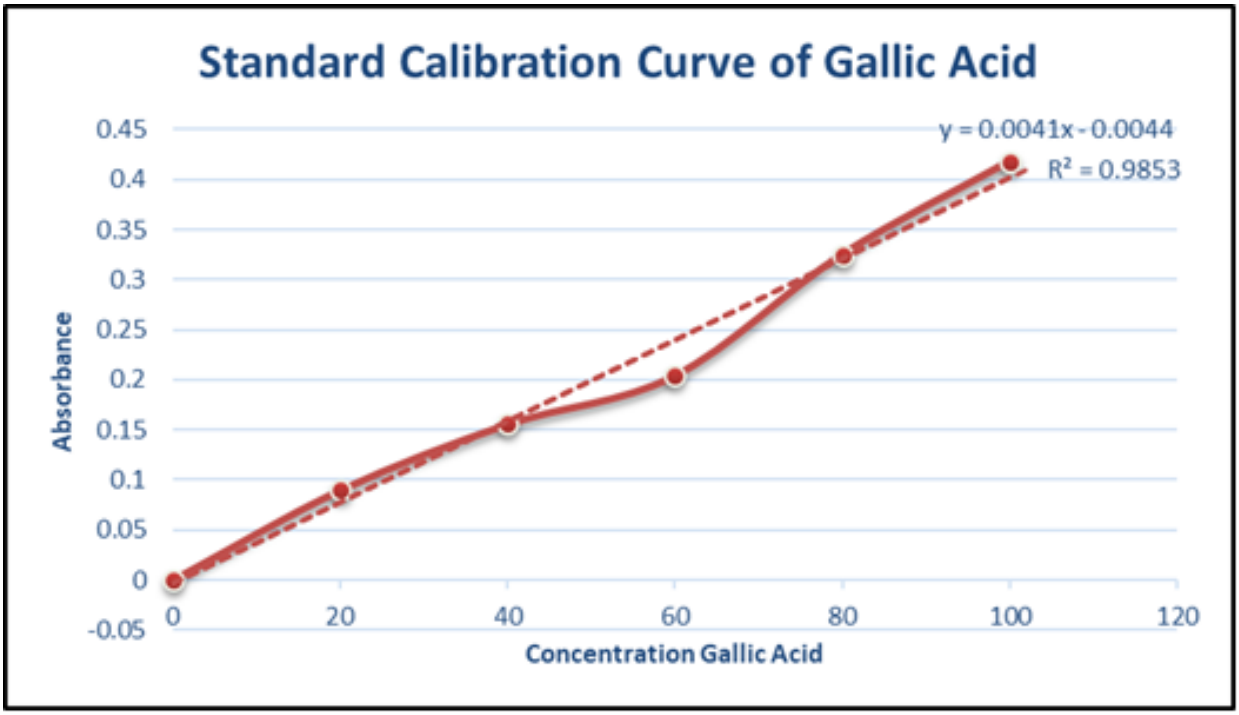

Figure 4: Standard calibration curve of Gallic Acid

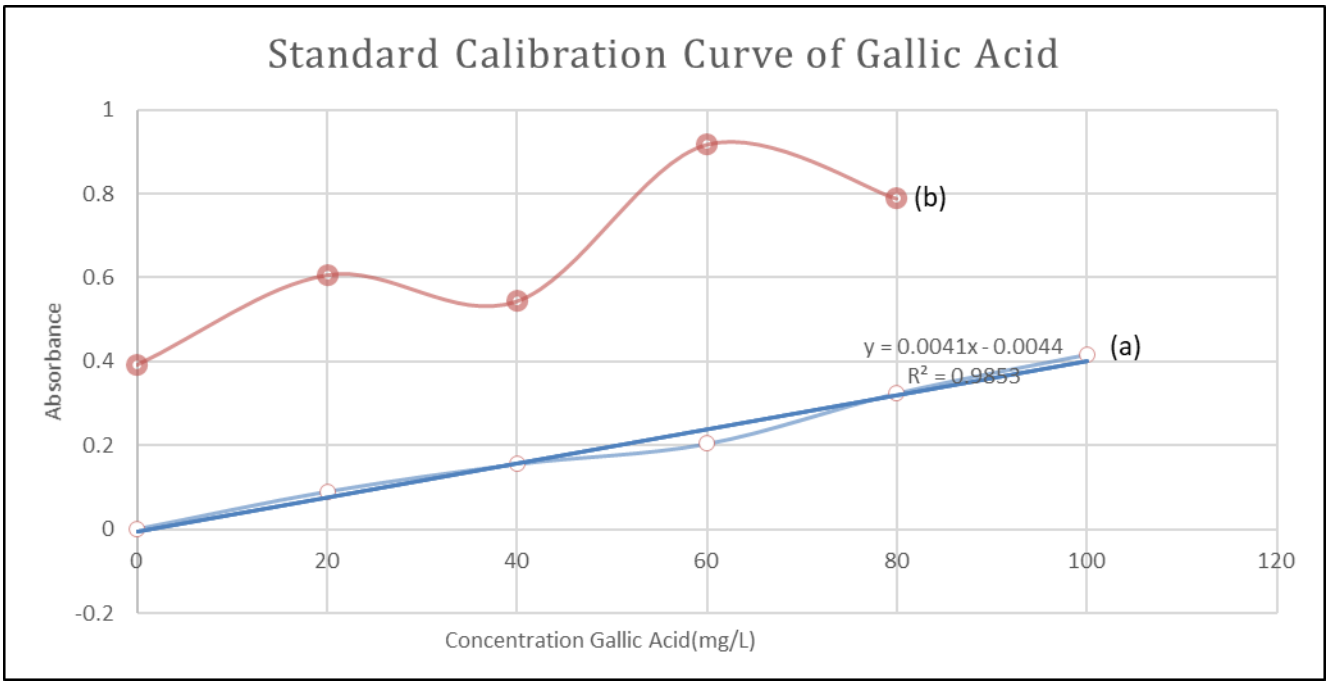

Figure 5: Standard calibration curve Gallic Acid (a) Standard calibration line (b) Ethanol

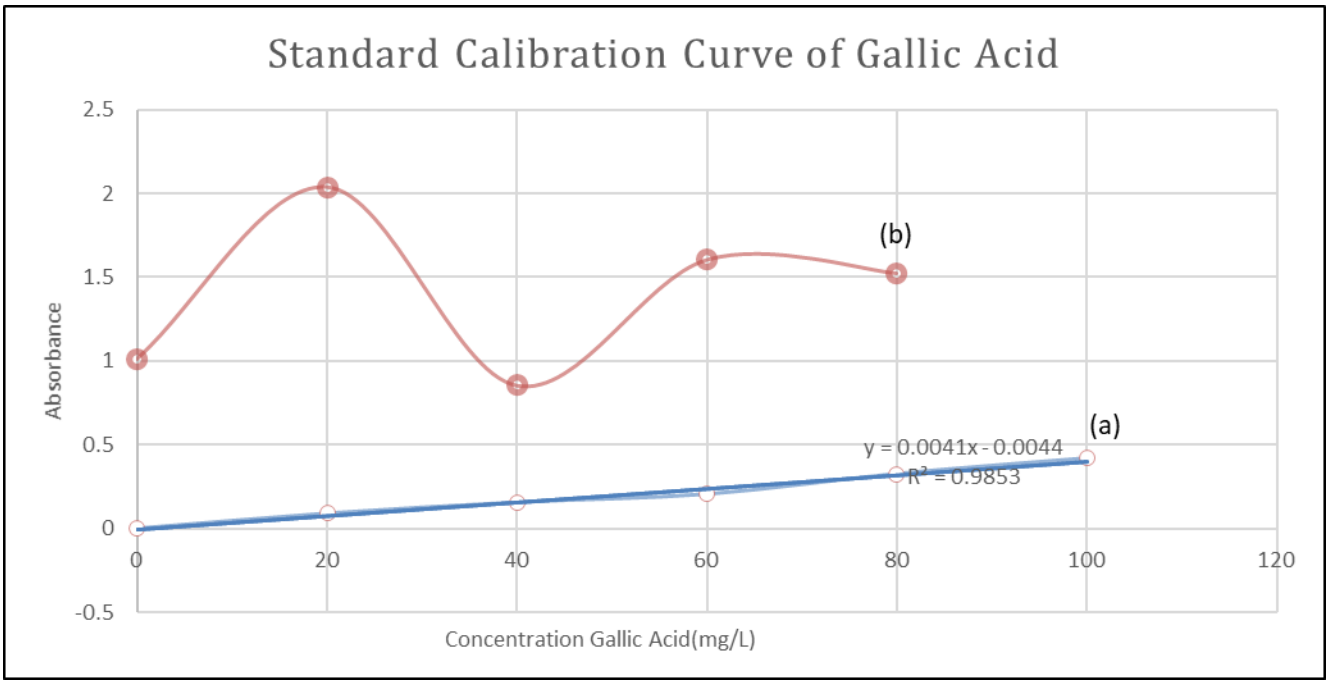

Figure 6: Standard calibration curve Gallic Acid (a) Standard calibration line (b) Methanol

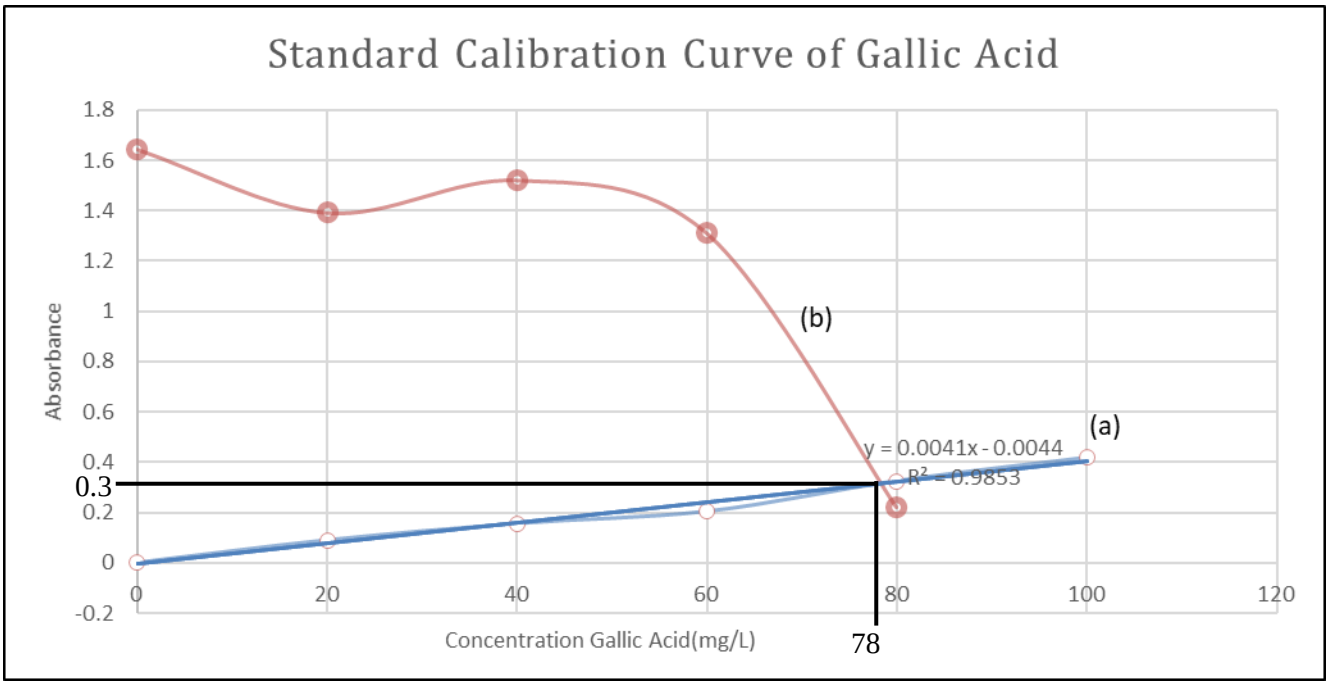

Figure 7: Standard calibration curve Gallic Acid (a) Standard calibration line (b) Acetone

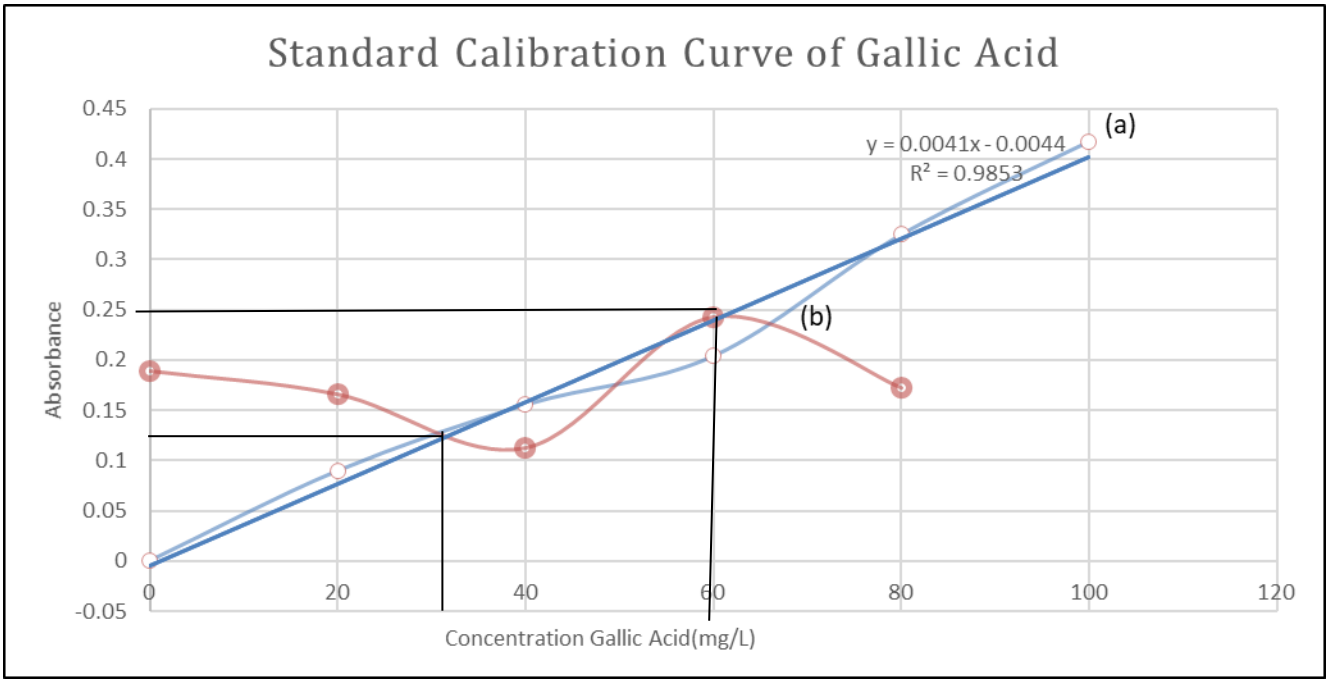

Figure 8: Standard calibration curve Gallic Acid (a) Standard calibration line (b) Ethyl Acetate

Conclusion:

Hibiscus rosa sinensis possess many benefits and it is known to be used as anti-fertility, anti-diabetic, antioxidant, anti-microbial, anti-inflammatory, and anti-pyretic activities. The Ultrasonic Assisted Extraction (UAE) methods is simple, rapid, environmentally friendly, and comprehensive. Table 1 shows the result of extraction yield by using Ultrasonic Assisted Extraction (UAE) method. The method of extraction is simple, inexpensive and convenient to use. Ultrasonic water bath facilitates the extraction of organic and inorganic compounds from solid matrices using liquid solvents. Sonication is the production of sound waves that create cavitation bubbles near the sample tissue, which break down to disrupt cell walls, thereby releasing cell contents. The highest weight after extraction is 102.95 g for methanol at time (30 min), while the lowest weight after extraction belongs to acetone at time (5 min, 15 min and 30 min) by 102 76 g and ethyl acetate at time (60 min).From the average of extraction yield in Table 1, methanol has the highest yield of extraction and the lowest extraction yield is ethyl acetate. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols. That is why, methanol has the highest extraction yield. The graph of the weight of sample after extraction and extraction yield by different solvent can be seen in Figure 2 and Figure 3. However, as for ethyl acetate, it has the lowest extraction yield but able to determine the phenolic content. Figure 4 shows standard calibration curve of Gallic acid where $Y= 0.0041 X – 0.0044$ and $R^2 = 0.9853$. After preparing standard calibration curve for Gallic acid, the sample is tested with UV-VIS spectrophotometer to determine the phenolic content. Figure 5 until Figure 8 shows the result of phenolic content by comparing it with standard calibration curve. Figure 7 shows the result of using acetone as a solvent to determine the phenolic content. At absorbance 0.3 and concentration at 78 mg/L the phenolic content can be determined by acetone. Figure 8 shows phenolic content test by using ethyl acetate as a solvent. From the graph, it shows that at concentration between 20 and 40 mg/L and at 60 mg/L the phenolic content is observed. As a conclusion, the yield of extraction does not affect the determination of the phenolic content.