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# Quantitative Test Of Quercetin Content Of Rome Beauty Apple Flour Extract Using The High-Performance Liquid Chromatography Method

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## Abstract

**Introduction:** Apple is a fruit containing antioxidant phytochemicals, one of which is quercetin, a flavonoid compound. Apples are one of the natural ingredients that have a beneficial effect on health. Rome beauty apples (*Malus sylvestris Mill.*) are natural antioxidants that are safer for consumption compared to synthetic antioxidants, which have the potential to cause harm. Oxidative stress occurs due to an imbalance in the amount of antioxidants in the body, causing cell damage. High-Performance Liquid Chromatography (HPLC) was conducted to determine the antioxidant content, especially quercetin, in Rome Beauty apple extract.
**Objective:** to find out how to quantitatively test the quercetin flavonoids from Rome Beauty apple extract (Malus sylvestris Mill.) using HPLC to determine the levels of quercetin flavonoids in Rome Beauty apple extract (*Malus sylvestris Mill.*).
**Method:** The method of sample preparation was carried out using a maceration extraction technique with 70% ethanol solvent. Determination of the quercetin content in Rome Beauty Apple Extract was carried out using the High-Performance Liquid Chromatography (HPLC) method and a UV spectrophotometer detector.
**Results:** The results of the quantitative tests that have been carried out conclude that Rome Beauty apple extract (*Malus sylvestris Mill.*) has a quercetin content of 557.999 ppm and a quercetin content of 0.557%.
**Conclusion:** Rome Beauty apples (*Malus sylvestris Mill.*) positively contain the flavonoid compound quercetin

**Keywords:** *Rome Beauty Apple, HPLC, Quercetin*

## Introduction

Fruit is a natural ingredient source containing various antioxidant compounds (Jideani *et al.*, 2021). Natural antioxidants are generally safer to consume than synthetic ones, which have potentially detrimental effects such as pro-oxidative properties (Liu, 2022). Apple is a fruit with abundant antioxidants (Asma *et al.*, 2023). Apples have beneficial effects against oxidative stress and related diseases (Oyenihi *et al.*, 2022).

Oxidative stress is a condition where there is an imbalance between free radicals and the amount of antioxidants in the body, causing cell damage (Oyenihi *et al.*, 2022). Oxidative stress can cause cancer, diabetes mellitus, neurodegenerative disease, cardiovascular disease, rheumatoid arthritis, kidney disease, and eye disease (Pisoschi *et al.*, 2021). Malang is the largest producer of apples in Indonesia, which have the potential to be a source of natural antioxidants. *Apples* are plants that originate in West Asia. This plant lives in areas with subtropical climates with cold temperatures and air conditions. Batu City is an expansion city of Malang Regency that produces many varieties of apples, one of which is the Rome Beauty apple, which has the potential to have properties as a natural antioxidant (Dohitra *et al.*, 2015). *Rome Beauty apples* are a natural ingredient that has beneficial effects on health. Rome Beauty apples contain catechin, epicatechin, phloridzin, allergic acid, chlorogenic acid, and quercetin, which act as antioxidants. One of the flavonoid compounds in quercetin, which has the potential as an antioxidant, has a working mechanism by reducing free radicals (Rusita *et al.*, 2019; Zaddana *et al.*, 2020). Quercetin is a flavonoid reported to exhibit various biological activities and is used for medical applications (Tronins *et al.*, 2023).

The potential pharmacological activity of flavonoids, especially quercetin compounds, is the basis for using High-Performance Liquid Chromatography (HPLC) to determine the quercetin content of Rome Beauty apple extract, as in previous research (Mizzi *et al.*, 2020). The purpose of this study was to find out how to quantitatively test the flavonoid quercetin from Rome Beauty apple extract (*Malus sylvestris Mill.*) using HPLC to determine the levels of the flavonoid quercetin found in Rome Beauty apple extract (*Malus sylvestris Mill.*).

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## Methods

***Design and Material***

This research is a descriptive observational study. This study focuses on the quercetin content of Rome Beauty apples (*Malus sylvestris Mill.*). This research was carried out in different laboratories; namely, the manufacture of Rome Beauty apple Simplicia was carried out at the UPT of the Materia Medika laboratory in Batu City Malang, and the manufacture of apple extract and analysis of the quercetin content was carried out in the Food Engineering laboratory of Soegijapranata Catholic University Semarang.

***Simplicity-making Process***

The study began with preparing the dry extract (simplicial), namely Rome Beauty apples washed thoroughly in running water and then sliced thinly. After that, it was dried in an oven at 50oC for three days. Then, dry Simplicia is obtained, crushed using a blender, and sieved to get uniform Simplicia particles.

**Rome Beauty Apple Extract Process**

The extract was prepared by maceration using 70% methanol. Simplisia powder weighed as much as 50 grams, dissolved in 250 mL of 70% methanol in a glass vessel, and stirred until homogeneous. The maceration process was carried out for five days. On the fifth day, filtering and remaceration were carried out by adding 250 mL of 70% methanol for two days with the same treatment. On the second day of repatriation, screening is carried out. The results of the filtering (filtrate) of maceration and maceration are mixed and evaporated using a vacuum rotary evaporator at a temperature of 500C until a thick extract is obtained. Pour it into a porcelain cup, heat it with an electric stove and with the help of a fan while continuing to stir, and keep the temperature controlled with a thermometer. This process evaporates ethanol to obtain a thick extract with a concentration of 100%. This maceration method is very advantageous because it is cheap and easy to do and does not damage compounds that cannot withstand heating. It is adjusted to the physical and chemical properties of the class of compounds in Rome Beauty apples (*Malus sylvestris Mill.*), namely the form of flavonoid compounds that are not heat resistant and easily oxidized at high temperatures (Setiani *et al.*, 2017).

**Quercetin Standard Solution Process**

The quercetin standard solution dissolves the standard with methanol and sonicating for 15 minutes, then filters with a 0.22 µm filter membrane. The results of making a standard quercetin solution are soluble and ready for analysis in a High-Performance Liquid Chromatography (HPLC) system.

**Standard and Sample Preparation**

The first standard preparation performed was 10.0 mg of quercetin standard. Then, the standard was dissolved in a 10 mL measuring flask with methanol solvent up to the mark (Quercetin content became 1 mg/mL or 1000 µg/mL). Then 1000 µg/mL of mother liquor was taken, and as much as 1 mL was dissolved in a 10 mL measuring flask with methanol solvent up to the mark (Quercetin level became 100 µg/mL). A standard curve was made from 100 µg/mL mother liquor and the solution with a 0.22 µL membrane filter. For sample preparation, weigh 25 mg of the sample, dissolve it in methanol up to 10 mL, and filter the sample solution using a 0.22 µm membrane filter. Next, inject 20 µL of sample filtration into the HPLC system. Repeat replication at least five times (Husnia & Budiarti, 2021)

## Results and Discussions

The sample characteristics consist of maternal age, maternal education level, and family income, which can be seen in the following table:

**Table 1.** Characteristic sample

| Subject Characteristic | Frequency n | Percentage % |
|---|---|---|
| Mother's Age | | |
| No Risk (20-35 th) | **21** | **70** |
| Risk (>35 th) | **9** | **30** |
| Gestational Age | | |
| First Trimester (4-13 weeks) | **7** | **23** |
| Second Trimester (14-26 weeks) | **21** | **70** |
| Third Trimester (27-40 weeks) | **2** | **7** |
| Mother's Education level | | |

| Subject Characteristic | Frequency n | Percentage % |
|---|---|---|
| Elementary School | **3** | **10** |
| Middle and High School | **24** | **80** |
| College | **3** | **10** |
| Family Income | | |
| Below the regionl minimum wage (<Rp2.000.000) | **24** | **80** |
| Above the regionl minimum wage (>Rp2.000.000) | **6** | **20** |

Based on **Table 1**, information was obtained that the majority of pregnant women were not at risk, as many as 21 people (70%), while nine people (30%) were pregnant women who were at risk (>35 years). Pregnant women with the majority of gestational age in the second trimester (14-26 weeks) were 21 people (70%), while in the first trimester, there were seven people (23%), and in the third trimester, there were two people (7% ). Most pregnant women had a secondary education level (SMP-SMA), as many as 24 people (80%). In comparison, the maternal education levels were primary and higher (university) as many as three people (10%). The majority of the mother's family had an income below the regional minimum wage (<Rp. 2,000,000), as many as 24 people (80%), while the family income was above the provincial minimum wage (>Rp. 2,000,000) as many as six people (20%).

**Table 2.** Analyzed Univariate Independent and Dependent Variables

| Independen and Dependen Variable | Frequency n | Percentage % |
|---|---|---|
| Iron Intake | | |
| Not enough | **24** | **70** |
| Enough | **6** | **30** |
| Vitamin D intake | | |
| Not Enough | **24** | **70** |
| Enough | **6** | **30** |
| Nutritional Status | | |
| Malnutrition | **24** | **70** |
| Good Nutrition | **6** | **30** |
| Anemia Occurrence | | |
| Anemic | **25** | **75** |
| Non Anemic | **5** | **25** |

Based on **Table 2.** Most pregnant women's food intake is deficient in iron, as many as 24 people (70%), while six people (30%) have sufficient iron intake in pregnant women. Most pregnant women's food intake of vitamin D is deficient, as many as 24 people (70%), while pregnant women with sufficient vitamin D intake are six people (30%). Most pregnant women experienced poor nutritional status, 24 people (70%), while six pregnant women experienced good dietary grade (30%). Most pregnant women also experience anemia, as many as 25 people (75%), while five people (25%) do not experience anemia

**Table 3.** Relationship between Iron Intake, Vitamin D, and Nutritional Status on the Incidence of Anemia Using Bivariate Analysis

| **Variable** | Anemia Occurrence | | | | **OR** | **CI 95%** | | $P^a$ |
|---|---|---|---|---|---|---|---|---|
| | **Anemic** | | **Not Anemic** | | | **Lower** | **Upper** | |
| | **n** | **%** | **n** | **%** | | | | |
| Iron Intake | | | | | 15,00 | 1,652 | 136,172 | 0,016* |
| Not enough | 19 | 45 | 5 | 25 | | | | |
| Enough | 4 | 20 | 2 | 10 | | | | |
| Vitamin D Intake | | | | | 0,427 | 0,033 | 4,228 | 0,417 |
| Not enough | 19 | 45 | 6 | 30 | | | | |
| Enough | 4 | 20 | 1 | 5 | | | | |
| Nutritional Status | | | | | 0,787 | 0,165 | 10,743 | 0,787 |
| Malnutrition | 19 | 45 | 5 | 25 | | | | |
| Good Nutrition | 4 | 20 | 2 | 10 | | | | |

$P^a$: *Chi-Square Test* *) *P<0.05 Significant* *n: number of people*

**Table 3** shows that iron intake is significantly related to the incidence of anaemia (OR=15, 95% CI, p=0.016), namely that 19 pregnant women (45%) who experienced insufficient iron intake resulted in anaemia. The OR value is 15, meaning inadequate iron intake can increase the risk of anaemia by 15 times compared to sufficient iron intake. Vitamin D intake was not significantly related to the incidence of anaemia (OR=0.427, 95% CI, p=0.417). Nineteen pregnant women (45%) who experienced insufficient vitamin D intake resulted in anaemia. The OR value is 0.427, meaning inadequate vitamin D intake can increase the risk of anaemia by 0.427 times compared to sufficient vitamin D intake. Nutritional status was not significantly related to the incidence of anaemia (OR=0.787, 95% CI, p=0.787); namely, 19 pregnant women (45%) who experienced poor nutritional status resulted in anaemia. The OR value is 0.287, meaning poor nutritional quality can increase the risk of anaemia by 0.287 times compared to good nutritional status.

**Table 4.** Relationship between Mothers age, Family Income, Mother's Education Level, and Gestational Age on the Incidence of Anemia Using Bivariate Analysis

| Variable | Anemia Occurrence | | | | OR | CI 95% | | *P*[a] |
|---|---|---|---|---|---|---|---|---|
| | Anemic | | Not Anemic | | | Lower | Upper | |
| | n | % | n | % | | | | |
| Mother's Age | | | | | | | | |
| No Risk (20-35 th) | 6 | 30 | 3 | 15 | 0,287 | 0,048 | 2,457 | 0,279 |
| Risk (>35 th) | 7 | 35 | 4 | 20 | | | | |
| Family Income | | | | | | | | |
| Below the regionl minimum wage (<Rp2.000.000) | 9 | 45 | 5 | 25 | 0,139 | 0,616 | 32,069 | 0,128 |
| Above the regionl minimum wage (>Rp2.000.000) | 4 | 20 | 2 | 10 | | | | |
| Mother's Education level | | | | | | | | |
| Elementary School | 2 | 10 | 1 | 5 | 1,009 | 0,240 | 4,233 | 0,419 |
| Middle and High School | 9 | 45 | 5 | 25 | | | | |
| College | 2 | 10 | 1 | 5 | | | | |
| Gestational Age | | | | | | | | |
| First Trimester (4-13 weeks) | 7 | 35 | 4 | 20 | 2,151 | 0,346 | 13,392 | 0,895 |
| Second Trimester (14-26 weeks) | 5 | 25 | 3 | 15 | | | | |
| Third Trimester (27-40 weeks) | 1 | 5 | 0 | 0 | | | | |

**) p 0.05 Significant n: number of people*

Based on **Table 4**, information was obtained that maternal age was not significantly related to the incidence of anaemia (OR=0.287, 95% CI, p=0.279), namely that six mothers (30%) were at risk of developing anaemia. Seven pregnant mothers (35%) are not at risk of anaemia. The OR value is 0.287, which means that the age of a mother at risk can increase the risk of anaemia by 0.287 times compared to that of a mother who is not at risk. Family income was not significantly related to the incidence of anaemia (OR=0.139, 95% CI, p=0.128). That is, nine pregnant women (45%) whose income was below the minimum wage (<Rp. 2,000,000) experienced anaemia, and as many as four pregnant women (20%) who earned above the minimum wage (>Rp. 2,000,000) experienced anaemia. The OR value = 0.139 means that mothers whose income is below the minimum wage can increase the risk of anaemia 0.139 times compared to mothers who earn above the minimum wage. The level of education was not related to the incidence of anaemia (OR=1.009, 95% CI, p=0.419). Namely, nine pregnant women (45%) had secondary education (middle and high school), and two pregnant women (10%) had essential (primary) and tertiary (college) education levels. An OR value of 1.009 means that pregnant women with a basic education level can increase the risk of anaemia by 1.009 compared to mothers with a higher education level. Gestational age was not significantly related to the incidence of anaemia (OR 2.151, 95% CI, p=0.895), namely seven pregnant women (35%) in the first trimester (4-13 weeks) and five pregnant women (25%) in the second trimester. (14-26 weeks), as many as one pregnant woman (5%) in the third trimester (27-40 weeks).

**Table 5.** Multiple Analyzed Independent Variable and Confounding

| Variable | B | Wald | Sig | OR | CI95% | |
|---|---|---|---|---|---|---|
| Nutrition status | -1.544 | 0.382 | 0.537 | 0.214 | Lower | Upper |
| Mother's age | -1.374 | 0.587 | 0.444 | 0.253 | 0.002 | 28.616 |
| Gestational age | 38.254 | 0.000 | 0.998 | 4.107 | 0.008 | 8.509 |
| Level of education | -0.281 | 0.020 | 0.889 | 0.755 | 0.000 | |
| Family income | 36.991 | 0.000 | 0.998 | 1.162 | 0.015 | 38.579 |
| Iron intake | -58.885 | 0.000 | 0.997 | 0.000 | 0.000 | |
| vitamin D intake | 16.935 | 0.000 | 0.999 | 22.64 | 0.000 | |

Pa: Multiple linear regression test *) p<0.05 significant

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Based on **Table 5,** it can be concluded that of all the independent variables (nutritional status, maternal age, education level, family income, iron intake, and vitamin D intake it is thought not to influence anaemia in pregnant women. The largest OR value was 22.64, meaning that vitamin D intake has a 22.64 times chance of causing anaemia in pregnant women.

Based on **Table 1,** information was obtained that the majority of mothers were not at risk (20-35 years) as many as 11 people (55%). Meanwhile, nine mothers are not at risk (45%). Maternal age < 20 years or above and > 35 years can cause anaemia. This is because iron consumption for 20 years is divided by the fetus in the womb and its biological growth, which, of course, still requires a lot of iron consumption (George *et al*., 2021). After the age of 35, they enter an early degenerative stage when body function is not optimal, and they have various health problems. Pregnancies under 20 years and over 35 years are pregnancies with a risk of anaemia (Bellakhal *et al*., 2019; Sunuwar *et al*., 2019).

According to Parischa *et al*. (2023), mothers of at-risk ages can reduce the incidence of anaemia by 0.68 times compared to mothers of non-risk ages. The results of this study show that there is conformity with the theory put forward by (Guo *et al*., 2022) that the ideal maternal age in pregnancy is the 20-35 year age group and at this age, mothers have healthy reproduction and are less at risk of pregnancy complications. The age group < 20 years is at risk of anaemia because reproductive development is not yet optimal, and according to Kumar and Lahiri (2023), pregnancy in the 35-year age group is associated with deterioration and decreased endurance as well as various diseases that often occur at this age. Pregnancy at >35 years of age is a high-risk pregnancy because, at this age, chronic health problems often occur, one of which is the risk of anaemia. Bleeding that occurs during childbirth, if not handled properly, will cause anaemia. Apart from that, age is not the only factor that causes anaemia. There are other factors, namely socio-economic factors (Kang *et al*., 2023).

Based on **Table 1**, information was obtained that the majority of mothers had a secondary education level (SMP-SMA), as many as 14 people (70%). Meanwhile, there were three mothers (15%) who had primary (primary) and higher (university) education. According to research by Edison (2019), the relationship between education level and the incidence of anaemia in pregnant women shows that the prevalence of anaemia in mothers who have a low level of education reaches 90.3% compared to mothers who have a high level of education, which is only 9.7%. The Chi-Square test results obtained a value of $\rho = 0.001$. This is due to the mother's lack of knowledge on how to process good food so that it does not damage the nutritional content in it and also due to low education, the mother does not work, which reduces household income, and the mother cannot buy nutritious food so that due to this limitation anaemia occurs (Panchal *et al* ., 2022). Based on interviews during the preliminary study, mothers only received education from health workers at the Community Health Center only once a month. So, the information obtained is lacking because, during the education class for pregnant women, only one theme is given, such as consuming blood supplement tablets regularly.

Based on **Table 1**, information was obtained that the majority of family income was below the minimum wage (<Rp. 2,000,000), as many as 14 people (70%). Meanwhile, six people (30%) had family income above the minimum wage (>Rp. 2,000,000). The prevalence of anaemia is greater in pregnant women with incomes lower than the Regional Minimum Wage (UMR). This affects the purchasing power of food families consume because >57% of family income is spent on purchasing food (Gibore et al., 2021; Pasricha et al., 2023). According to Septiasari (2019) research, it was found that 25 out of 39 pregnant women (61.0%) of mothers with income < minimum wage experienced anaemia, while among pregnant women with income ≥ minimum wage, there were 16 out of 47 people (39.0 %) have anaemia. The results of the chi-square statistical test show that the p-value = 0.005 ($p \leq 0.05$), RP 3.460 (95% CI = 1.421 – 8.425), so it can be concluded that mothers with an income <UMR increase the incidence of anaemia by 34 times compared to mothers with an income >UMR causes mothers not to get adequate nutrition, thereby risking anaemia. The number of families will influence the food distribution within the family. The lack of family income causes a reduction in the location and purchase of daily food, thereby reducing the quantity and quality of the mother's food per day, which has an impact on reducing nutritional status. A common nutritional disorder in pregnant women is anaemia. The food sources needed to prevent anaemia generally come from protein sources, which are more expensive and difficult for those with low incomes. This deficiency increases the risk of anaemia in pregnant women and accelerates the risk of morbidity in mothers (Mekonen & Alemu, 2021; Maugliani & Baldi, 2023). Based on preliminary study interviews, as many as 65% of pregnant women said they consumed two pieces of vegetable protein (tempeh, tofu, processed nuts) 3-4 times per week.

Based on **Table 1,** information was obtained that the majority of pregnant women with poor nutritional status were 14 people (70%). Meanwhile, there were six pregnant women with good nutritional status (30%). Steven et al. (2022) stated the relationship between nutritional status and the incidence of anaemia. *Nutritional status* is also defined

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as health status resulting from a balance between nutrient needs and input and is a basic need for pregnant women. Nutritional status and the incidence of anaemia were also shown by Dewi and Mardiana (2021), who found that the risk of anaemia in pregnant women was 2.9 times higher for pregnant women with poor nutritional status than those with good nutritional status. This comparative figure has a large role in influencing the health of pregnant women. The estimated determinant of R2 is 0.047, meaning that nutritional status contributes 4.7% in influencing the incidence of anaemia. Even though the contribution value is small, as long as the regression coefficient $\beta1$ is not statistically zero, scientifically, it can be proven that there is an influence between nutritional status and the incidence of anaemia (Guo *et al*., 2022)

Based on **Table 1**, information was obtained that the majority of mothers' iron intake was less than 14 people (70%). Meanwhile, six people (30%) had sufficient iron intake, according to research conducted in Jatinangor District regarding the consumption patterns of pregnant women, which shows that the intake of food sources of iron (Fe) in pregnant women is still inadequate, namely 53 people (93%) out of 57 pregnant women (Putri *et al*., 2020). This happens because the source of iron consumed does not come from heme, such as meat and animal food sources, so it is not easy to absorb and does not support the presence of iron in the body. The same thing was done in research in TuaTunu Pangkal Pinang Village regarding the relationship between nutritional intake and the incidence of anaemia in preconception women, which showed that the intake of food sources of iron (Fe) in preconception women was still insufficient, namely 98.4% (Devriany & Wardani, 2019). This is because the consumption of less balanced foods can interfere with the absorption of iron in the body, and the majority of respondents consume more vegetable protein than animal protein. In addition, the lack of consumption of side dishes, which are a source of iron which is useful during pregnancy for the formation of new haemoglobin, compensates for the small amount of iron which is constantly excreted by the body (especially through urine, faeces and sweat), and replaces iron losses during pregnancy—lactation for milk secretion (Devriany & Wardani, 2019).

Mothers who are malnourished are at risk of having a difficult or long labour, giving birth to a baby prematurely (not yet full term), bleeding in the mother after giving birth and usually during labour, the mother also lacks the strength to push during the labour process so giving birth using a high surgical procedure for pregnant women lack nutrition (Xu *et al*., 2022). There are 41% of pregnant women suffer from malnutrition. The emergence of nutritional problems in pregnant women, such as the incidence of CED, cannot be separated from the social, economic, and biosocial conditions of pregnant women and their families, such as education, income level, food consumption, age, parity and so on (Andersen *et al*., 2022). Malnutrition can cause the mother to suffer from anaemia; the blood supply that delivers oxygen and food to the fetus will be hampered, so that the fetus will experience impaired growth and development. Therefore, monitoring the nutrition of pregnant women is very important (Nuru *et al*., 2021).

Based on **Table 1**, it was found that the majority of mothers' vitamin D intake was less than 14 people (70%). Meanwhile, six people (30%) had sufficient vitamin D intake. Vitamin D is a vitamin that is needed for various metabolic processes in the body. It is fat-soluble and is produced by human skin with energy obtained from food intake. Vitamin D deficiency in pregnant women has an impact on the fetus and newborn. The prevalence of vitamin D deficiency is 63% in Indonesia and Malaysia. According to Smith M. Ellen and Tang Pricha Vin (2019), vitamin D can increase erythropoiesis by increasing the proliferation of erythroid progenitors and reducing proinflammatory cytokines. Additionally, by decreasing proinflammatory cytokines that stimulate hepcidin and through direct transcriptional regulation of the HAMP gene, vitamin D can suppress hepcidin expression. Reducing proinflammatory cytokines and hepcidin may increase iron bioavailability for erythropoiesis and haemoglobin synthesis by restoring iron recycling, preventing iron absorption in macrophages, and eliminating impaired iron absorption, thereby protecting against anaemia.

## Conclusions

Iron intake is significantly related to the incidence of anaemia ($p=0.016<0.05$) and can increase the risk of anaemia 15 times. Meanwhile, vitamin D intake is not significantly related to the incidence of anaemia ($p=0.417>0.05$). Nutritional status is not significantly related to the incidence of anaemia ($p=0.787 >0.05$). Factors that have a significant influence on anaemia are family income and gestational age. There is a need for further research regarding interviews using recall to determine the food ingredients consumed by respondents and education using questionnaires regarding anaemia in pregnant women.

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