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Extraction of Protein from Moringa oleifera Leaves for Facial Mask Production

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ABSTRACT

The use of facial mask is spreading wide all around the world due to its health benefits in skincare. There are many cases of cosmetic or beauty products containing ingredients that are not suitable for the skin where the pH reading increases or decreases due to either too acidic or alkaline. This research is conducted in order to determine the protein content in Moringa oleifera leaves and skincare product for the production of halal-certified facial mask. The extraction and determination of protein content are conducted using salting-out method with ammonium sulfate and by Bradford assay method. The extraction of protein from the leaves is done by using different weight of leaves, whereby the results for all samples are compared. This research is also conducted to produce a facial mask from the extracted protein from Moringa oleifera leaves using a suitable and the best formulation. Randomly selected respondents were asked to fill out a questionnaire to assist in this study. The survey was conducted using the 'Google Form' application as a platform. The formulated facial mask and the facial mask available in the market are then taken for a few parameter analyses. Final results showed the average protein content percentage in Moringa oleifera leaves is 33.33% and 16.67% in facial mask. The pH value for Moringa oleifera leaves is recorded to be in the range of pH 5.0- 6.0. The pH value for Moringa oleifera leaves is pH 5.01 and pH 5.29 for facial mask. A total of 45 respondents participated in the questionnaire analysis and 91.1% of them were women. A total of 68.9% were facial beauty masks users.

Keywords: Moringa oleifera; protein extraction; facial mask; toxicity analysis; quantitative analysis

INTRODUCTION

Moringa oleifera Lam. is the scientific name for a plant with common names such as drumstick tree, ben oil tree and miracle tree (Ahmad et al. 2014). This plant belongs to the Moringaceae family and is physically small with of only 5- 10 meters in height and with soft wood and white in color (Sujatha and Poonam, 2017). Moringa oleifera grows in the Middle East, Africa and in Asia such as Malaysia and Indonesia. This plant also grows well in the West East India and Philippines (Leone et al. 2015). This plant has been widely used for traditional medicine, while the flowers and the leaves of Moringa oleifera are used as edible herbs (Raja et al. 2016). There are many researches proving that the leaves of this plant have potential in helping the healing process for wounds (Lim et al. 2019). The leaves contain high nutrition especially the level of protein which is the highest in 100 grams leaves (Raja et al. 2016). Other plant parts such as wood barks and seeds are often used to produce oil in Ayurveda medicinal treatment (Paikra et al. 2017).

Facial masks are used worldwide including Malaysia and a lot more Asian countries and they are especially popular amongst women. There are three major types of facial mask which are sheet mask, peel-off mask and wash-off mask (Nilforoushzadeh et al. 2018). Each of the facial masks type has its own formulation and different ingredients are used depending on the ingredients' characteristics and the main function it gives to the skin (Himaja et al. 2015). Amira (2017) mentioned that cosmetics can be placed into a few catagories. Table 1 shows the different category of cosmetics.

TABLE 1. Categories of cosmetics

Category	Function	Main product
Skincare	Cleansing	Face cleansing foam and cream
	Conditioner	Lotion
	Protection	Moisturizing lotion
Make up	Base	Face powder
	Pointer	Eye shadow
Body care	Bathing	Soap
	Protection from sunlight	Sunscreen cream

Source: Amira (2017)

One of the cosmetic categories in skincare products is facial mask (Amira 2017). The use of facial mask may help in getting rid of pimples, scars, anti-aging and black spots as stated in Ayurveda treatment (Himaja et al. 2015). There are three main types of facial masks which are sheet mask, peel-off mask and wash-off mask (Nilforoushzadeh et al. 2018). All the three facial mask types are produced using different formulations (Nilforoushzadeh et al. 2018). The main ingredients for the facial mask formulation are usually extracted from either animals, herbs or even fruits (Nilforoushzadeh et al. 2018). The production of each facial mask is different in terms of quality and other materials, though they may use the same main extracted ingredients as the main function of the product (Suhery and Anggraini 2016). Collagen is one of the most common and most popular main ingredients for facial mask production (Shu et al. 2014). Other main ingredients for the formulation of facial masks are using clay and herbs as the main extracted substance (Formulary Face Mask 2017; International Trade Administration 2016).

Protein can be found in all organic matters, though in different amounts for different kinds of matter (Comerford and Pasin 2016). The extraction of protein can be conducted in the laboratory by using the right methods. The choosing of extraction method is extremely important especially when dealing with plants due to the many compounds that may disrupt the extraction process (Abdullah et al. 2017). The most applied method in extraction of protein is by salting-out using ammonium sulfate due to its high solubility, and high ionic strength, besides being cheaper and easier to obtain in pure form (Duong-Ly and Gabelli 2014). By salting-out, the precipitation of protein will be formed (Duong-Ly and Gabelli 2014).

There are a few methods that can be used in determining the protein content of samples. The most common method in the laboratory used by researchers especially by pharmaceutical researchers is Bradford method (Vilhena et al. 2015). This is because of the easy access of its protein standard for the standard curve which is Bovine Serum Albumin (BSA) (Nouroozi et al. 2015).

Protein is one of the most important elements in cosmetic products including in facial mask (Nilforoushzadeh et al. 2018). The source of collagen extraction, which is one of the famous ingredients for facial masks can be rather questionable whether they came from non-halal animals, and thus the extraction of protein from herbs or plants is guaranteed in terms of halal sources. Quantitative analysis research can be conducted with regards to the general knowledge of this particular topic (Jamil et al. 2019).

Islam is the main religion in Malaysia and is practiced by the majority of the population (Siti and Mohd, 2015). Halal is defined as anything and everything that is guaranteed its cleanliness without any suspicious or questionable items or substances and it is allowed in Islam by referring to Syariah law and fatwa (Draft Malaysian Standard, 2018). The concept of halal is always an issue with food and drinks (Siti and Mohd 2015). Nevertheless, Harlida and Azhar (2017) stated that the concept of halal is not only limited to food and drinks but also in all aspects including cosmetic products. According to Draft Malaysian Standard (2018), halal cosmetic products for Muslims are already standardised. Products withholding a halal certificate are indeed safe to be used.

Cosmetic products also possible having the risk of damaging skin, skin infections and other complications such as immunity unstable (Nieradko - Iwanicka et al. 2017). One of the methods in determining pH value of a sample is by using pH sensor film but this is not being recorded as a common practice in the laboratory (Amdah et al. 2019). The determination of pH value can be conducted in the laboratory using pH meter (Perugini et al. 2018). Additionally, pH buffers can be used for saponification and formulation thickening by neutralising the thickener (Mohammad et al. 2017).

In studying the knowledge of facial mask users, the method usually used is the quantitative method which is by survey or questionnaire analysis. There are a lot of research that have been using this method on any subjects (Jamil et al. 2019).

METHODOLOGY

In order to conduct the research and experiment, there are a few things that need to be considered such as the methods used, materials, apparatus and analysis methods. There are few steps to be followed accordingly so that the flow of the research goes smoothly.

EXTRACTION OF PROTEIN AND DETERMINATION OF PROTEIN CONTENT

There are a few steps to be taken before the experiment can be conducted. Each step is to be done using the right procedures to ensure the safety such that the success of the experiment can be achieved. Figure 1 shows the flowchart for this experiment.The procedures are adopted from Abdullah et al. (2017) and Nouroozi et al. (2015). After the samples are collected, standard curve is plotted using Bovine Serum Albumin (BSA) as standard protein. The preparation of the samples is conducted in 1.5 mL centrifuge. Standard samples are to be left for 5 - 10 minutes after centrifuge. Each sample is then taken to UV- spectrophotometer for evaluation of optical density (OD) at wavelength of 595 nm. Based on OD values, the standard curve can be plotted.

The preparation of buffer tris (hydroxymethyl) aminomethyl- hydrochloric acid (Tris-HCl) includes tris (hydroxymethyl) aminomethane, hydrochloric acid and distilled water. Tris(hydroxymethyl)aminomethane is first inserted in a 100 mL Duran bottle and 80 mL of distilled water is added into the same Duran bottle and the solution is then stirred. After stirring, by using pH meter to monitor the pH value change, hydrochloric acid is added gradually until pH value reached 8.0, which is the pH value required for this buffer.

The extraction of protein is conducted using salting-out method with ammonium sulfate. Samples that are to be tested are Moringa oleifera leaves and facial masks available in the market. Different weights of leaves are used for this experiment; 20 grams, 40 grams and 60 grams. Moringa oleifera samples are dried in a conventional oven at temperature of 50^{o} Celsius until the moisture of samples reached below 10% and the samples are then grinded into powder form. Afterwards, 5 mL buffer is added for 20 grams leaves, 10 mL buffer for 40 grams leaves and 15 mL buffer for 60 grams leaves. The final step is the filtration process to obtain the protein precipitation and the steps are repeated over again for other facial mask samples.

The determination of protein concentration or content of samples is conducted using Bradford method. The 0.1 mL precipitated sample is added to 0.9 mL ammonium sulfate to be centrifuged for 5 minutes. The centrifugal speed is in the range of 5000 - 7000 rpm. The supernatant formed is then mixed slowly with 1 mL of distilled water. The 0.5 mL of solution is added to 2.5 mL Bradford reagent and left for 5 minutes. The optical density (OD) of samples is taken at wavelength of 595 nm and the steps are repeated for other facial mask samples.

FORMULATION OF FACIAL MASK

Table 2 and 3 show the formulation of sheet facial mask and wash-off mask to be produced.

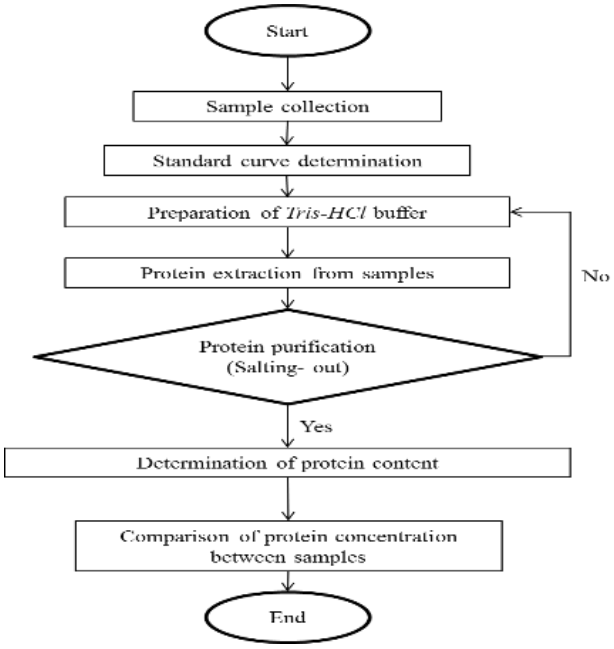

FIGURE 1. Flowchart for extraction of protein and determination of protein content

DETERMINATION OF PH VALUE

The pH values of Moringa oleifera formulated mask and the facial mask in the market are to be determined using pH meter in the laboratory. The calibration of pH meter is conducted using neutral pH buffer (7.0) and acidic pH buffer (4.0). The pH meter electrode is cleaned using distilled water and wiped off using tissue before the electrode can be dipped into samples for determining the pH value of the samples (Perugini et al. 2018).

QUANTITATIVE ANALYSIS

This analysis is conducted to evaluate facial mask users regarding the products used in terms of buying habits and knowledge of the products. Figure 2 shows the flowchart for the analysis.

TABLE 2. Formulation of sheet facial mask

Ingredient	Total content
Protein extract	5g
Castor oil	3g
Tween 80	5g
Glycerin	5g
Ethylene glycol	2.5g
Sodium EDTA	4.0g
Oleum citri	5 drops
Demineralized water	75.5g

Source: Reveny et. al (2017)

TABLE 3. Formulation of wash- off facial mask

Ingredient	Content in 100 g (%)
M. oleifera leaves powder	30
Honey	20
Rose water	20
Lemon oil	20
Tea tree oil	10

Source: Yadav and Yadav (2017)

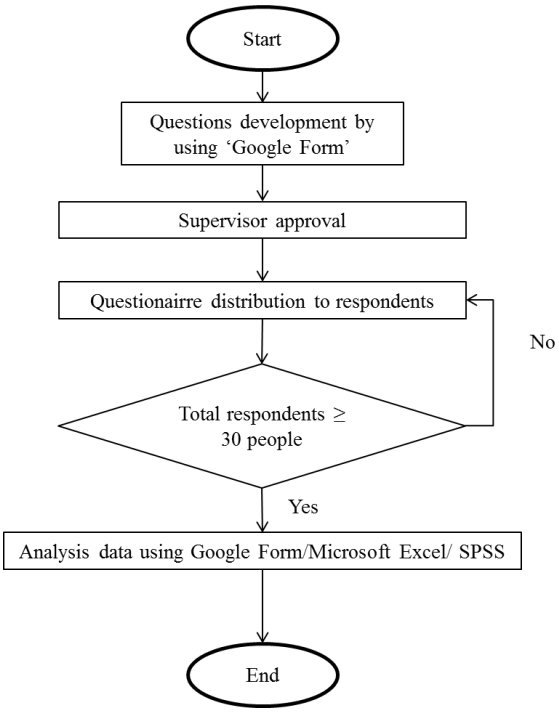

FIGURE 2. Flowchart for quantitative analysis

RESULTS AND DISCUSSION

In this research, the samples used are *Moringa oleifera* leaves and facial masks samples. There are six facial masks samples of which two of them are facial masks produced in the laboratory using suitable formulation and the other four are collected from various markets.

EXTRACTION OF PROTEIN AND DETERMINATION OF PROTEIN CONTENT

The method used to obtain the values for standard curve is Bradford assay using Bradford reagent and salting-out technique using saturated ammonium sulfate. *Moringa oleifera* leaves are collected at Taman Pinggiran, Batu Caves, Selangor and the samples are brought to the laboratory for further study. The leaves are then dried in a conventional oven at temperature of 50°Celsius until the samples' moisture value reached below 10%. With moisture value of 8.881% as the samples' final moisture, the leaves samples are then grinded into finer size before proceeding to the next study. Figure 3 shows the grinded *Moringa oleifera* leaves.

Facial mask samples are collected and labelled properly before proceeding to the next study. Figure 4 shows the six facial mask samples and Table 4 shows the category and halal certified statement of facial mask samples. Protein is extracted from the samples by using buffer Tris-HCl and after that the determination of protein content in each sample is conducted. Figure 5 shows chart of protein content against mass for *Moringa oleifera* leaves samples whereas protein content in facial mask samples is depicted in Figure 6. The percentage of protein content in each sample is shown in Table 5.

FIGURE 3. Moringa oleifera leaves powder

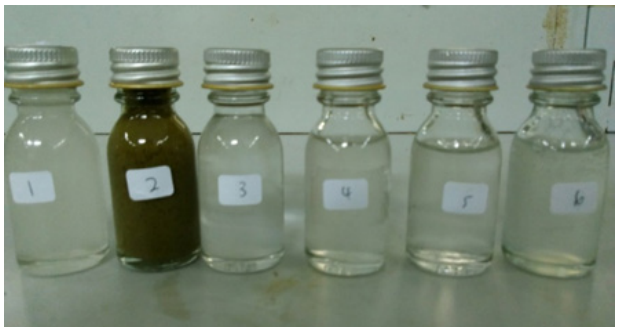

FIGURE 4. Facial mask samples

TABLE 4. Category of facial mask samples

Facial mask label	Category	Halal certified
1	Facial mask from *M.oleifera* (Sheet)	-
2	Facial mask from *M.oleifera* (Wash-off)	-
3	Local	Yes
4	Not local	Yes
5	Not local	No
6	Collagen	No

TABLE 5. Protein content percentage

	Moringa oleifera leaves	Facial mask					
		1	2	3	4	5	6
Average protein content (g/L)	0.92	0.1726	0.1499	0.16	0.155	0.1563	0.2103
Protein content percentage (%)	33.33	17.19	14.93	15.93	15.44	15.57	20.94

TABLE 6. Comparison protein content percentage with previous research

	Moringa oleifera leaves	Reference	Facial mask	Reference
Current result (%)	33.33		16.67	
Previous research (%)	24.85	Mohamad et al. 2019	13.59	Perugini et al. 2019
Error (%)	34.12		22.66	

The results show that the amount of protein content in Moringa oleifera leaves are larger than facial mask samples. For Moringa oleifera leaves samples, the protein contents in samples are analysed to increase with the increase of the mass of the samples. This result is obtained due to the different amount of nutrient with different mass of leaves. The protein content of 100 grams Moringa oleifera leaves is recorded to be 9.40 grams (Raja et al. 2016). The protein content percentage for Moringa oleifera leaves is 33.33% and the average protein content percentage for facial mask samples is 16.67%. The protein content in facial mask is considerably lower than the leaves samples because the protein in organic material tends to denature during the production process if there is slight condition difference such as temperature and way of handling (Comerford & Pasin, 2016). Table 6 shows the results of protein percentage with previous research.

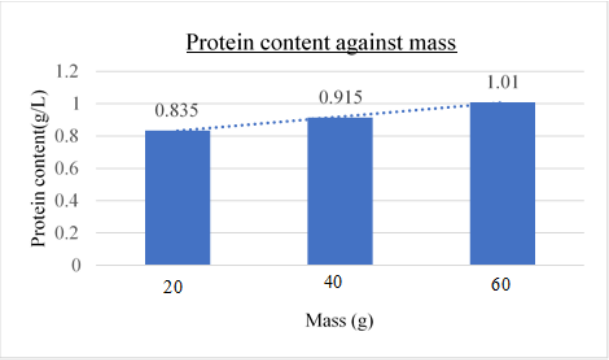

FIGURE 5. Protein content in Moringa oleifera leaves

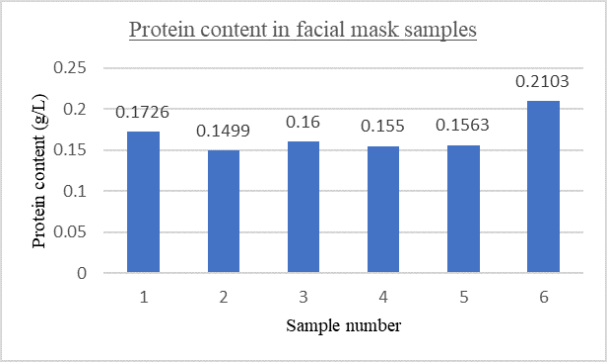

FIGURE 6. Protein content in facial mask samples

TABLE 7. pH value

	Moringa oleifera leaves	Facial mask					
		1	2	3	4	5	6
pH value	5.01	5.3	5.1	5.5	5.45	5.5	5.21

TABLE 8. Comparison of pH value result with previous research

	Moringa oleifera leaves	References	Facial mask	References
Current result	5.01	This Study	5.29	This Study
Previous research	4.66	Foline et al. 2011	5.12	Perugini et al. 2018

PH VALUE DETERMINATION

The pH value for each sample is determined using pH meter. This test is done as pH is one of the most important and common parameters to be determined for toxification test on cosmetics products (Mohammad et al. 2017). From previous research, it is recorded that the most suitable pH

value of human skin is in the range of pH 5.5 to 6.0 and cosmetic products have to be in the range of pH 5.0 to 8.0 in order to be consumed in the market (Reveny et al. 2017).

The results of pH values are shown in Table 7. The results showed that all samples are in the range of pH 5.0 to 8.0. The average pH value of all samples is pH 5.29 which means that all of the samples are suitable for humans' skin. Table 8 shows the comparison of current result with previous research.

QUANTITATIVE ANALYSIS

Quantitative analysis is done using Google Form application. There is a total of 45 respondents whom participated in this research. There are four parts or sections of questions in the questionnaire; Section A, Section B, Section C and Section D. Section A consists of basic questions about respondents, Section B consists of questions about the frequency of facial mask use, Section C consists of questions about knowledge in production of facial mask and Section D consists of questions regarding Moringa oleifera.

The results that have been analysed and showed that 91.1% of the respondents are facial masks users. There are three most commonly applied facial masks; sheet, wash-off and peel-off masks are the most popular. The sheet mask is found to be the most applied between the three types. A lot of respondents usually used facial mask products from overseas instead of that produced in Malaysia. This might be because the production of facial mask in Malaysia is limited and some of them are expensive. Majority of the respondents have no knowledge of the existence of Moringa oleifera but is willing to try out any cosmetic products containing ingredients from Moringa oleifera.

CONCLUSION

This research is conducted in order to achieve four objectives. The right and suitable formulations for facial masks produced in the labs have been applied to both sheet facial mask and wash-off facial mask. A total of six facial mask samples have been taken for analysis in this research. Four of them are samples taken from the market and the other two are produced in the laboratory with the right formulations. Analysis in extracting and determining the protein content in all the samples are conducted by using buffer Tris-HCl for the extraction, salting-out using saturated ammonium sulfate and by Bradford assay for determination of protein content. The results showed the percentage of protein content in Moringa oleifera leaves is 33.33% which is higher than the average percentage of protein content in facial mask samples with the value of 16.67%. For toxification test, all of the samples are taken for determination of pH value using pH meter. The pH value obtained showed the value for all the samples to be in the range of pH 5.0 -6.0, which is also in the range of pH values for human skin and cosmetics. Quantitative analysis has been participated by 45 respondents with 91.1% of all the respondents are female as analysed in Part A. Part B showed that 35.5% of the respondents never used wash-off facial masks, 40.6% used sheet facial mask at least once in a fortnight, 53.1% never used peel-off facial masks, 40.6% used products from fruits and herbs and 50.0% used products from collagen and others. Analysis in Part C of the questionnaire showed 43.8% of the respondents always recognise product brands before buying, 37.5% never used local products, 40.6% used non-local products at least once a week, 40.6% recognise halal products and 34.4% really understand the effects of facial masks on the skin. Analysis in Part D showed that many of the respondents do not know the existence of Moringa oleifera either in Malaysia or in other countries.

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DECLARATION OF COMPETING INTEREST

None

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