PROGRESS IN ENGINEERING APPLICATION AND TECHNOLOGY
e-ISSN: 2773-5303

PEAT

Vol. 5 No. 1 (2024) 698-708
https://publisher.uthm.edu.my/periodicals/index.php/peat

The Effect of Size Reduction and Different Oil Ratio on Microemulsion of *Trigonella Foenum-Graceum* and *Phyllanthus Embilica* Oils and Their Antioxidant Properties

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DOI: https://doi.org/10.30880/peat.2024.05.01.075

Article Info

Received: 28 December 2023
Accepted: 18 January 2024
Available online: 15 June 2024

Keywords

Fenugreek seed oil, amla seed oil, ultrasonication, micro-emulsion, size reduction, antioxidant activities, physicochemical properties

Abstract

This study aims to develop a hair tonic using micro-sized fenugreek and amla seed oils to treat premature greying of hair (PGH). The prevalence of this problem in young people is concerning, and existing solutions like hair dye have limitations. Fenugreek and amla seed oils possess antioxidant and hair-beneficial properties, and size reduction for both oils can enhance their effects. Ultrasonication will be used to create micro-sized oils, then tested for physicochemical properties, antioxidant activities, and the storage stability. Young people are increasingly facing hair issues like premature greying, yet current solutions like expensive transplants and potentially harmful dyes leave them wanting a much cheaper and safer solution. Existing haircare products often contain concerning ingredients, and even PGH treatments like dyes may have negative effects. The aim to use ultrasonication method, a technique used in drug formulations, is to create stable combined fenugreek and amla seed oils micro-emulsions for hair products, potentially providing a safer and more effective solution for hair problems like PGH. The method of the research is to use ultrasonication technique to reduce the particles size of the oil emulsion into smaller size ranging into the micro-sized region (0.7 to 700 micrometers), and to determine the particle size, Zeta Potential Analyzer (ZetaSizer) is used. From the result obtained, the second formulation for 1:1 ratio of fenugreek to amla seed oils which has higher water content, has the most significant size reduction of 88.93%. In conclusion, ultrasonication method does promises the size reduction for oil emulsions, and the result could be enhanced by adding centrifuge, and sieving method to further purify the oil emulsion creating a smaller particle size.

Progress in Engineering Application and Technology Vol. 5 No. 1 (2024) p. 698-708

1. Introduction

Presently, the world has been swarming with youths by the age of 30, in which 12% of women and 25% of man having hair-related problems such as hair loss and premature hair graying [1]. From this case study, a high prevalence of hair graying involved the teenagers where it could lead to loss of confidence and self-esteem in this group of people, prompting a quick or better solution for this problem.

Trigonella foenum-graecum (fenugreek) is a plant that has been used for centuries for its medicinal properties. For example, the seed of the fenugreek plant is used as traditional medicinal herb in India, China, Thailand, and South-East Asian countries [2]. It is rich in vitamins, minerals, and antioxidants, such as flavonoids, alkaloids, and saponins, and has been effective in treating hair related problems [3]. The second plant in this study, *Phyllanthus emblica* (Amla) has been studied for its benefits for example, the juice from the bark of the amla tree is mixed with honey and turmeric and used to treat gonorrhea [4]. In addition, amla contains calcium, Vitamin C, phosphorus, iron and much more nutrition [4]. Due to the aforementioned factors, combining amla into formulation for hair tonic could be an excellent idea as it might help in preventing premature hair greying.

The nanosizing technique refers to a process that reduces the particle size down to submicron range which can be done using several methods such as ultrasonication, micro fluidization, and high-pressure homogenization. However, these methods have their own limitations. For example, micro fluidization is much more complex and requires specialized equipment [5]. In this work, ultrasonication technique is chosen to produce nanosized oils for ultrasonication method does not involve thermal process and only uses ultrasound to break down particles of fenugreek and amla seed oils into nanosized particles. Ultrasonication is also much safer, cleaner, and affordable compared to high pressure homogenization and micro fluidization technique [6].

Hence, in this work, the focus of the study is to produce a formulation that could achieve micro-sized particles. The ultrasonication method will be used as the basis for production of micro-sized particles of the oil mixture by breaking down the oil particles and observed using Zeta Potential Analyzer (ZetaSizer). Additionally, the physicochemical properties and the antioxidant activities of the nano emulsified will be observed. Finally, is to assess the stability of the oil emulsion formulation.

2. Materials and Methods

2.1 Materials

Fenugreek seeds (India), Ultrasonic probe (Straits Scientific, Malaysia), Fourier Transform Infrared Spectroscopy (Thermo Scientific Nicolet iS5), Folin-Ciocalteu reagent, UV-Vis Spectrophotometer, 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2.1 Fenugreek and Amla Seed Oils

The fenugreek, *trigonella foenum-graceum* seed oil was obtained from supplier in Negeri Sembilan, Malaysia. Two bottles of 50mL of fenugreek seed oil make into 100mL in total volume. For amla *phyllanthus embilica* seed oil was obtained from supplier in Kelantan, Malaysia. A bottle of 100mL amla seed oil was obtained. Both oils will be mixed to prepare the oil phase before adding water phase into the mixture to produce emulsion. There will be three different ratios of fenugreek to amla seed oils which are 1:1, 1:2, and 2:1 produced. Three different tables of data, **Table 2.1 (a)**, showing the formulation done for non-ultrasonication, **Table 2.1 (b)** shows the First Formulation for emulsion of fenugreek to amla seed oil with three different ratios and amount of emulsifier added into the mixture. And **Table 2.1 (c)** shows the second formulation for emulsion of fenugreek to amla seed oil with three different ratios and amount of emulsifier added into the mixture.

Table 2.1 (a) *Table of formulation done for non-ultrasonication.*

Fenugreek to amla seed oil ratio	Fenugreek Seed Oil (ml)	Amla Seed Oil (ml)	Distilled water (ml)	Total mixture volume (mL)	Amount of emulsifier (Soy Lecithin) (g)
1:1	15.00	15.00	20.00	50.00	0.400
1:2	10.00	20.00	20.00	50.00	0.536
2:1	20.00	10.00	20.00	50.00	0.600

Table 2.1 (b) *First Formulation for emulsion of fenugreek to amla seed oil with three different ratios and amount of emulsifier added into the mixture.*

Fenugreek to amla seed oil ratio	Fenugreek Seed Oil (ml)	Amla Seed Oil (ml)	Distilled water (ml)	Total mixture volume (mL)	Amount of emulsifier (Soy Lecithin) (g)
1:1	10.00	10.00	10.00	30.00	0.194
1:2	5.00	15.00	10.00	30.00	0.194
2:1	15.00	5.00	10.00	30.00	0.194

Table 2.1 (c) *Second Formulation for emulsion of fenugreek to amla seed oil with three different ratios and amount of emulsifier added into the mixture.*

Fenugreek to amla seed oil ratio	Fenugreek Seed Oil (ml)	Amla Seed Oil (ml)	Distilled water (ml)	Total mixture volume (mL)	Amount of emulsifier (Soy Lecithin) (g)
1:1	15.00	15.00	20.00	50.00	0.400
1:2	10.00	20.00	20.00	50.00	0.536
2:1	20.00	10.00	20.00	50.00	0.600

2.2 Ultrasonication Method

Ultrasonication method for fenugreek seed oil is handled at amplitude of 5 for around 7 minutes to 10 minutes with the on/off setting set to 5 seconds and 10 seconds respectively. This is to ensure that the fenugreek seed oil is at nanosized particles state while maintaining the temperature of the nano emulsion of the oils to remain at optimal temperature of 35℃.

2.3 Determination of Nano Sized Formulated Oils.

Testing and characterising the size of nanoparticles is made possible by the Zetasizer. It is a well-known and often used dynamic light scattering (DLS) technology that offers useful details regarding the stability and size distribution of nanoparticles in a liquid dispersion (7). Dynamic light scattering, commonly referred to as photon correlation spectroscopy, is the underlying principle on which the Zetasizer runs. In this method, a sample of nanoparticles is exposed to a laser beam, and the variations in scattered light intensity brought on by the Brownian motion of the particles are then measured. Following that, the size distribution and further characteristics of the nanoparticles are determined by analysing these intensity fluctuations.

2.3 Physicochemical Properties Analysis

2.3.1 FTIR Analysis

It is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. FTIR spectroscopy works by passing infrared radiation through a sample. The molecules in the sample absorb some of the infrared radiation, and the amount of radiation that is absorbed depends on the chemical bonds in the molecules. By measuring the amount of infrared radiation that is absorbed at different wavelengths, an FTIR spectrometer can create a spectrum that is unique to the sample.

2.3.2 pH Test

To do the pH testing, the pH meter was turned on and let it to show “READY” on the display. After that, the pH meter probe was calibrated inside a beaker filled with buffer solution with specific pH value which are 7.0 for neutral, and 11.0 for basic pH value. Before testing the on the sample, the pH meter probe was rested inside a distilled water to test either the probe was fully functional or does not show the wrong pH value. Right after the pH meter calibration, wipe the probe of the pH meter with paper towel before proceeding the testing with the fenugreek and amla seed oil emulsions samples. For the sample oil emulsions samples testing, only 10mL was used for the pH value testing. The pH probe was submerged inside the sample until the pH meter shows a pH reading. The probe was let submerged until the value on the pH meter shows consistent readings.

Progress in Engineering Application and Technology Vol. 5 No. 1 (2024) p. 698-708

2.4 Antioxidant Activities Testing

A variety of procedures and methodologies are used in antioxidant testing to evaluate a substance or sample's antioxidant capacity or activity. Antioxidants are substances that shield cells and tissues from free radical damage, which can result in oxidative stress and several health problems. The efficiency of antioxidants in scavenging free radicals and averting oxidative damage is evaluated using a variety of antioxidant testing techniques. These techniques include the DPPH Radical Scavenging Assay, and the Total Phenolic Content (TPC) assay. The scavenging activity is assessed by DPPH test, and concentration is assessed by TPC.

2.4.1 DPPH Assay

With minor modifications, the radical scavenging activity of DPPH was assessed. The presence of an antioxidant changed DPPH's purple colour to yellow. 1 mL of DPPH radical solution (0.04 mg/mL in methanol), 1 mL of fenugreek extracts and 3 mL of methanol were used in the test. After 30 minutes kept in the dark, the absorbance of the residual DPPH was measured using a spectrophotometer at 517 nm. The measurement was done three times

2.4.2 Total Phenolic Content (TPC) Test

Total phenolic content (TPC) was measured using the Folin–Ciocalteu reagent in each sample. A spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian) was used to take sample and standard readings at 765 nm against the blank sample (sample 1). 80 µL of the test sample was blended with 6 mL of water and 0.4 mL of Folinphenol Ciocalteu's reagent with 0.2 M. After 5 minutes, 1.2 mL of saturated sodium carbonate (Na2CO3) solution (7.5% w/v in water) was added to the mixture. The reaction was held in the dark for 2 hours before measuring the absorbance of the blue hue from separate samples at 765 nm. The TPC value of the fenugreek extract samples will be expressed as milligram of gallic acid equivalent per gram of dry weight sample (mg GAE/g).

2.5 Storage Stability Test

Storage stability test was done to observe the stability of the three formulations done for the fenugreek and amla seed oils emulsion. The storage stability test was conducted by storing the samples inside a contained dark space for around one week to see the condition of the formulated oil emulsion. After a week, the oil emulsions were observed to see either it separates or does it keep it emulsions form.

3. Results and Discussion

This chapter discussed the results of testing described earlier in Chapter 3. This chapter are divided into three part which are Determination of the nano sizing, followed by the physicochemical properties test, and determination of antioxidant activities.

3.1 Determination of the Size Reduction

To see whether the formulation of oils done achieve nano particles sizes, Zeta Potential Analyzer (ZetaSizer) was used to observe the sizes of the samples.

3.1.1 Formulation without using Ultrasonication.

The research started with the formulation done for the mixture of fenugreek and amla seed oils without the process of ultrasonication. This part of the research is to obtain the baseline size of the emulsion of the fenugreek and amla seed oil before running the emulsion for ultrasonication processes. The expected size of the emulsion without ultrasonication will be larger compared to the ultrasonicated formulation.

3.1.2 First Formulation with Less Water Content

For the result of the first formulation, the three-formulation ratios were observed using ZetaSizer to see if the nanoemulsion of fenugreek and amla seed oil were successfully obtained into nano size. In the first formulation, the use of ultrasonication process, using ultrasonicator, used to break down the oil mixture The significance size reduction can be observed in the graph for the three different ratios of fenugreek to amla seed oil emulsion for non-ultrasonicated and ultrasonicated formulations. The data difference for both non-ultrasonicated and ultrasonicated formulation can be thoroughly observed in Table 3.1.2 that shows the percentage reduction

between both formulations. To further visualize the data obtained, a graph was constructed as shown in Figure 3.1.2 that shows the size for both non-ultrasonicated and ultrasonicated and the size reduction value.

Table 3.1.2 *Data for the z-average for non-ultrasonicated and ultrasonicated oil emulsion and the percentage of the size reduction.*

	Z-average for non-ultrasonicated oil emulsions (d. nm)	**Z-average ultrasonicated (First Formulation) oil emulsions (d. nm)**	**Percentage Reduced (%)**
Ratio 1:1	5.284e4	2.535e4	52.02
Ratio 1:2	5.429e4	2.371e4	56.33
Ratio 2:1	1.616e4	9464	41.44

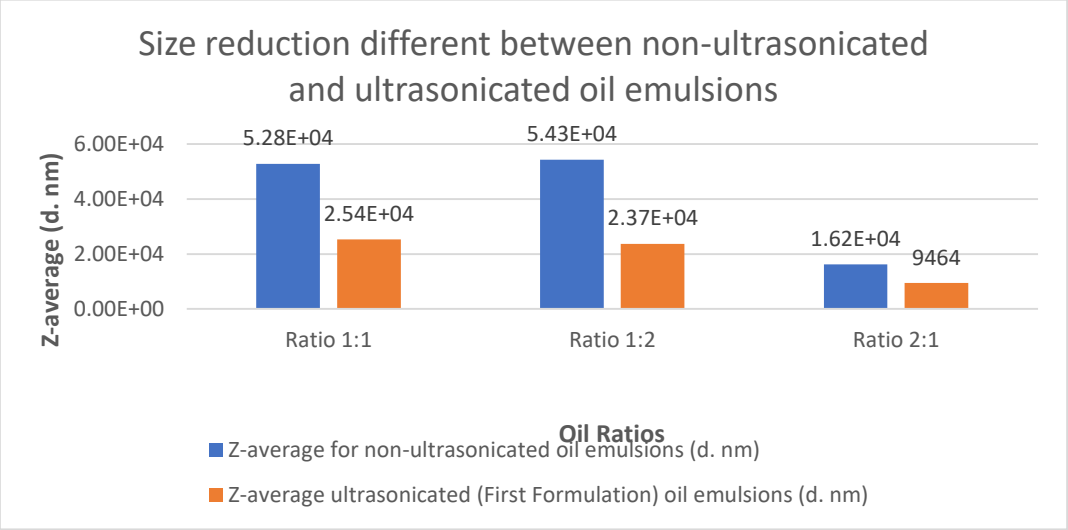

Fig. 3.1.2 *The overall reading for formulation of ratio 1:1 without ultrasonication process*

3.1.3 Second Formulation with Higher Water Content

In this study, a second formulation for the oil were done due the first formulation constitutes of a lower water content. Causing the formulation to be too thick and the zeta potential analyser cannot measure the size due to exceeding the size limit. In the second formulation, it was done in hope to reach nano size particles of the oil's micro emulsion process. The result of the size reduction can be seen in Table 4.1.3 and the visualization of the data can be seen in Figure 4.1.3. In the second formulation, it was hoped that the formulation would create a much smaller particles size in comparison to the first formulation as the second formulation have a much higher water content compared to the first formulation.

Table 3.1.3 *Data for the z-average for non-ultrasonicated and ultrasonicated oil emulsion and the percentage of the size reduction.*

	Z-average ultrasonicated (First Formulation) oil emulsions (d. nm)	**Z-average ultrasonicated (Second Formulation) oil emulsions (d. nm)**	**Percentage Reduced (%)**
Ratio 1:1	2.535e4	2805	88.93
Ratio 1:2	2.371e4	1.050e4	55.71
Ratio 2:1	9464	1.544e4	-63.144

Progress in Engineering Application and Technology Vol. 5 No. 1 (2024) p. 698-708

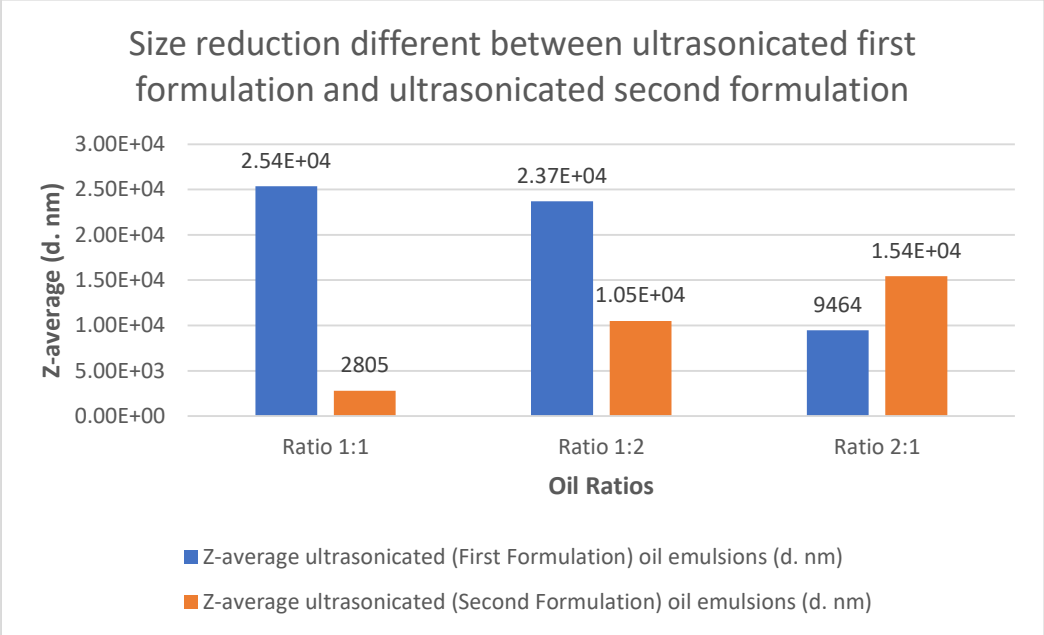

Fig. 3.1.3 *The graph to show the size reduction difference between first formulation and second formulation ultrasonicated oil emulsions.*

From the data obtained from the table and graph, it can be seen that the second formulation have a better formulation compared to the first formulation due to the higher water content. It helps in producing smaller particles size of oil emulsions for up to 88.93% in size reduction. But for the ratio 2:1, the size increases which is not a desired outcome as it should be reduced in size as shown from the 1:1 and 1:2 ratio. This could be caused by the oils might not be thoroughly emulsified during the mixing and the ultrasonication process. To overcome this inconsistent result, the Other than that, the size reduction for the second formulation is much better than the first formulation.

3.2 Physicochemical Properties Test

Molecular properties (such as molecular weight, dipole moment, polarizability, van der Waals volume, and surface area) and bulk properties (such as acidic or basic character in solution, octanol/water partition coefficient, solubility, etc.) are the two categories into which physicochemical qualities can be divided (8).

3.2.1 FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful analytical technique that can provide valuable insights into the physicochemical properties of fenugreek and amla seed oil, both individually and within a nanoemulsion formulation. For this reason, this study uses this method to obtain the properties of the nanoemulsion of fenugreek and amla seed oil. In Figure 4.3.1, a graph showing the transmittance from the FTIR spectrometer for each of the formulation of different ratio 1:1, 1:2, and 2:1. The overlay of the graph makes the analysis much easier and clearer as the peak for the different ratios can be differentiated and be compared from each other.

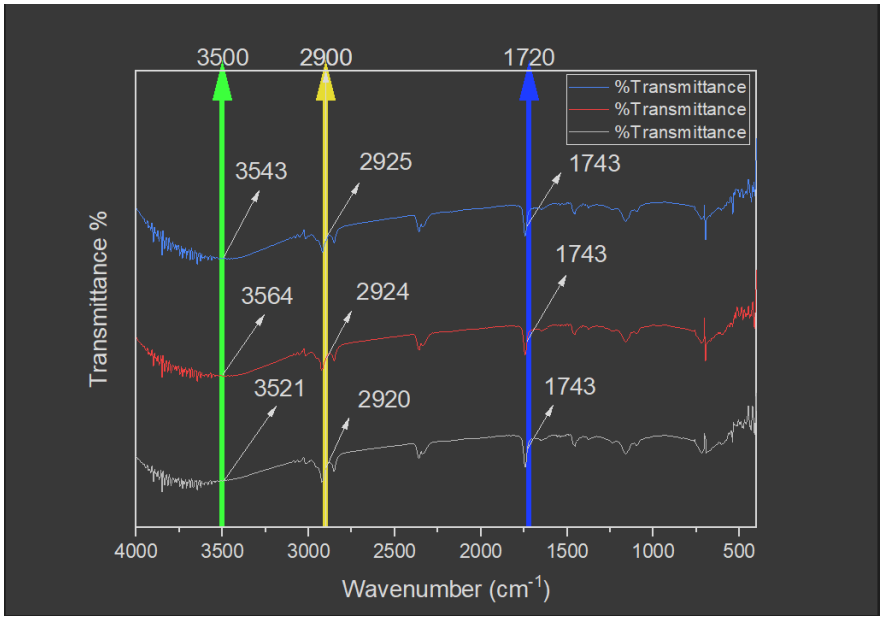

Figure 3.2.1 *Data compiled for FTIR for the three formulation 1:1, 1:2, and 2:1 showing the three main component peaks for the formulation.*

From the figure, three main peaks were chosen to represent the valuable bonding that contain the main benefits of either fenugreek seed oil, amla seed oil or for the nanoemulsion for both oils. At the first peak which is 1720 cm^{-1}, the three different ratio shows a peak at 1743 cm^{-1} which reflects the presence of carbonyl stretching vibration of the functional groups of triglycerides, C=O. This bonding function as the main storage form of fatty acids in fenugreek seeds oil and from the figure, from all the formulation done, the presence of this bond is consistent meaning it does not break up. Triglycerides are a type of lipid molecule composed of three fatty acids esterified to a glycerol backbone. Each fatty acid chain is linked to the glycerol through an ester bond, where an oxygen atom bridges a carbon atom from the fatty acid and a carbon atom from the glycerol (8). The presence and intensity of the ester carbonyl peak provides valuable information about the abundance and composition of triglycerides in fenugreek seed oil.

As for the second peak which is at 2900 cm^{-1}, the region in between at 2800~3000 cm^{-1}, is the fatty acid region inside the formulation. At this peak, shows the presence of hydroxyl group (O-H) that was derived from the presence of O-H stretching vibrations in the carboxylic acids, characteristics of fatty acids, like oleic acid, linoleic acid, and linolenic acid (9). The broadness of the peak is not just a random observation. It reflects the diverse nature of the fatty acids present. Each fatty acid exhibits slightly different O-H stretching frequencies depending on the length and degree of unsaturation of their carbon chains. In fenugreek seed oil, the presence of oleic acid (monounsaturated), linoleic acid (diunsaturated), and linolenic acid (triunsaturated) contributes to the broad expanse of the peak (9).

For the last chosen peak which is at 3500 cm^{-1}, range of 3200~3600 cm^{-1}, contains the hydroxyl (O-H) group. The presence of hydroxyl group derives from the presence of phenolic compounds such as gallic acid, and ellagic. These abundances of antioxidants are derived from the amla seed oil (Wongsa, P., et al 2022). This broad peak arises from the O-H stretching vibrations within the hydroxyl group (-OH), a functional group commonly found in phenolic compounds (10). In amla seeds, these phenolic compounds, particularly gallic acid and ellagic acid, are major contributors to the observed peak. The abundance of O-H groups within their structures collectively amplifies the peak, highlighting their significant presence.

3.2.2 pH Analysis

The pH value is very important as the formulation was intended for application to treat hair related problems. Therefore, having the formulation at an optimum value will help in enhancing the benefits of using the products and helps to prevent more damages towards the hair and the follicles. As each person has different skin conditions, it also applies towards their hair. At different pH value could help in different part of the hair, for example, at acidic pH of around 4.5~5.5, This range mimics the natural pH of healthy hair and scalp, helping maintain cuticle closure and shine. It's suitable for normal to oily hair, as it can help control sebum production and prevent frizz. Whereas, at neutral pH of 6.0~7.0, is generally tolerated by most hair types and offers a balance between cleansing and maintaining moisture. It can be a good starting point for versatile formulations. The aim of the research is to maintain a neutral pH as it has less harmful effects towards human hair and skin. The result of pH value measured

Progress in Engineering Application and Technology Vol. 5 No. 1 (2024) p. 698-708

is done for the second formulation of fenugreek and amla seed oil, this is due to the second formulation shows a much more stable nano emulsion**.** Table 3.3.2 shows the observed pH value for each different ratio of fenugreek to amla seed oil. Based on the data of pH value obtained, the most suitable is 1:1 ratio of fenugreek to amla seed oil ratio as it the closest to neutral pH compared to ratio 1:2 and 2:1.

Table 3.3.2 *pH values for second formulation of fenugreek and amla seed oil.*

Fenugreek to amla seed oil Ratio	pH value
1:1	5.55
1:2	5.23
2:1	5.23

3.3 Determination of Antioxidant Activities

3.3.1 DPPH Analysis

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a popular and widely used method for evaluating the antioxidant activity of various substances. It's a simple, quick, and inexpensive technique that relies on the ability of antioxidants to scavenge free radicals (11). Therefore, this method is suitable for determining the presence of antioxidants inside the formulation of the oils. For the DPPH assay, the testing was done on the second formulation as it shows the most promising result compared to other formulations done throughout the project. From Table 3.3.1 (a), showing the data obtained from the DPPH assay analysis.

Table 3.3.1 (a) *Data obtained from DPPH assay analysis for the three ratios of fenugreek to amla seed oil.*

Ratio of Fenugreek to Amla Seed Oil	**Reading 1**	**Reading 2**	**Reading 3**	**Average**
Blank	0.0000	0.0000	0.0000	0.0000
1:1	2.3149	2.1703	2.1317	2.2056
1:2	0.4565	0.4617	0.4556	0.4579
2:1	1.1448	1.1302	1.1293	1.1348

From **Table 3.3.1 (a)**, the inhibition percentage of DPPH (%), can be calculated by using the formula:

$$\%DPPH = \frac{Absorbance\ Control - Absorbance\ Sample}{Absorbance\ Sample} * 100$$

By using the formula, a data was tabulated in **Table 3.3.1 (b)** for the inhibition percentage of DPPH.

Table 3.3.1 (b) *The inhibition percentage of DPPH for three ratios of fenugreek to amla seed oil for absorbance at 517 nm.*

Ratio of Fenugreek to Amla Seed Oil	Absorbance at 517nm	Inhibition Percentage of DPPH (%)
Blank	0.0000	0.0000
1:1	2.2056	22.40
1:2	0.4579	74.59
2:1	1.1348	37.02

From the data obtained using the formula, the highest inhibition percentage of DPPH is for ratio 1:2 which inhibit 74.69% of the DPPH. For ratio 1:1 and 2:1, the inhibition percentage of DPPH is lower compared to ratio 1:2 and the inhibition percentage for 1:1 ratio is the lowest at only 22.40. This means that for ratio 1:1, it allows the absorption of antioxidants the highest making it the most suitable formulation that could carry the greatest number of antioxidants.

3.3.2 Total Phenolic Content (TPC) Analysis

A class of tiny molecules known as phenolic compounds is distinguished by the presence of at least one phenol unit in their structures (12). The diverse group of secondary plant metabolites known as phenolic compounds comprises phenolic acids, flavonoids, lignins, lignans, stilbenes, and tannins (13). The Folin-Ciocalteu (FC) assay, commonly referred to as the Total Phenolic Content (TPC) assay, is a popular method for estimating the total antioxidant capacity of samples containing phenolic compounds. While not as specific as other antioxidant assays like DPPH, this method was selected due to its simplicity, as it works almost the same as DPPH assay. Then TPC assay is also a versatile method as it can be used to test many types of samples ranging from food products to even cosmetic products. From Table 3.3.2 (a), it can be seen the data obtained from the TPC assay.

Table 3.3.2 (a) *Data for TPC assay analysis for the three ratios of fenugreek to amla seed oil.*

Ratio of Fenugreek to Amla Seed Oil	Reading 1	Reading 2	Reading 3	Average
1:1	0.9517	0.9605	0.9672	0.9598
1:2	0.3008	0.3113	0.2973	0.3031
2:1	0.4065	0.4024	0.3989	0.4026

From Table 3.2.2 (b), there are three different formulations for fenugreek and amla seed oil which are 1:1, 1:2, and 2:1. As observed from the table, the formulation that has the highest concentration of gallic acid is the ratio 1:1 which is 0.5962 microg/MI followed by formulation for ratio 2:1 at 0.2500 microg/MI and finally at the lowest concentration of gallic acid is 1:2 ratio formulation. This shows that amla seed oil has a higher phenolic content compared to fenugreek as for 1:2 ratio formulation, the concentration of gallic acid absorbed is the lowest. To visualize the data, a bar chart was constructed as shown in Figure 3.3.2 (b).

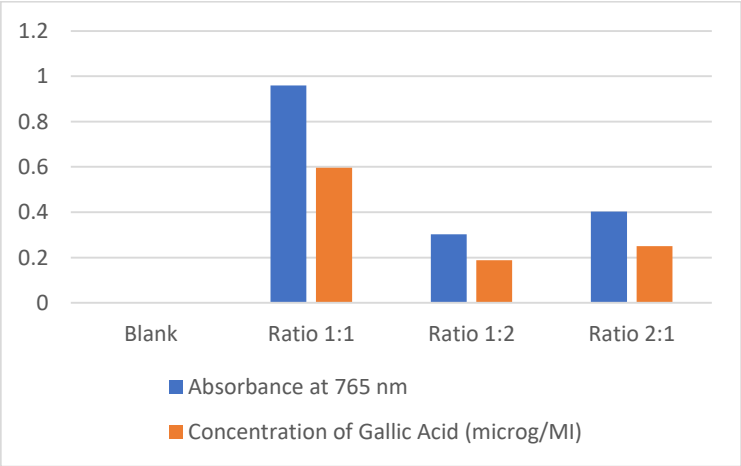

Figure 3.3.2 (b) *The difference between the concentration of gallic acid and absorbance at 765 nm between three different formulations.*

3.3.3 Storage Stability Test

This test was done to investigate the condition of the formulation after being stored inside a dark space for a week to see whether the formulation retains its composition, does not separated, or does it separate after being stored for a week. As can be seen in Figure 3.3.3, the three different ratios of oil emulsions.

Progress in Engineering Application and Technology Vol. 5 No. 1 (2024) p. 698-708

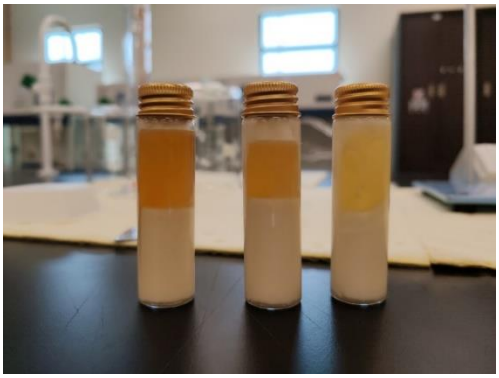

Figure 3.3.3 *Three conditions of the different ratios of oil emulsions, from the left is 1:1, 1:2, and 2:1 ratio.*

As observed from the storage stability test, the oil does separate after being stored for a week. This could be affected by insufficient emulsifier. Emulsifiers act as stabilizers, preventing the oil and water from separating. If the emulsion doesn't have enough emulsifier, it will become unstable and prone to separation over time. Another factor is that the temperature difference might affect the stability of the emulsion. The emulsion process involves the use of ultrasonication which is high in temperature, not exceeding 35°C, therefore during the storage phase, the temperature might drop and causes the emulsion to be unstable and causes visual separation between the oil phase and the water phase as shown in Figure 3.3.3.

4. Conclusion

From the study done on the effect of size reduction and oil ratio on micro emulsion of fenugreek and amla seed oils using ultrasonication method, it can be concluded that fenugreek had a lot of beneficial components for hair growth and health. The same goes for amla which have important antioxidant and vitamin to help treating hair related problem. By combining both fenugreek and amla seed oils into one formulation, it would further enhance the properties of the hair tonic produced to have both properties. For objective 1 which is to produce micro sized fenugreek and amla seed oils using ultrasonicator, it was achieved as observed from the size observed using Zeta Potential Analyzer. For the second objective which focuses on assessing the physicochemical properties and antioxidant activities of the nanosized oils. From the result of the physicochemical properties and antioxidant activities, the result of FTIR shows the peak of the bioactive component such as triglycerides for oil storage, hydroxyl group that made up into phenolic compounds such as gallic acid. As for the antioxidant activities of the micor-emulsion, the DPPH assay and TPC assay test shows a reaction meaning that there are presence of antioxidants and free radicals. As for the final objective which is to assess the stability of micro emulsified oils and to the determine the most suitable oil ratio for applications. The formulation that was done during the research shows that the formulation requires more water content than oil content to improve the efficiency of the oil emulsion and to help in the stabilization of the oil formulation.

5. Recommendation

For future research suggestion, there are a few key things to consider to be able to achieve the main objective of this research which are:

1) Formulate the formulation with higher water content than oil content.
2) Use buffer to redirect heat to help during the nano-sizing process using ultrasonicator.
3) After ultrasonication process, the sample could be centrifuged and further sieve to obtain even smaller particles.
4) Use different kinds of emulsifier to obtain better oil emulsion and more stable oil emulsions.

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