Phytochemical Screening and Chemical Analysis of Freeze-dried *Lawsonia inermis* Leaves Extract

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Abstract. *Lawsonia inermis* or Henna is proven to give a natural dye to skin and hair. However, there is still a lack of studies evaluating the properties of Henna in terms of its phytochemical composition. Thus, this study aims to extract henna leaves using the freeze-dried method before testing the phytochemicals in the extract. This study also seeks to analyze the presence of chemicals in the plant extract. The Thin Layer Chromatography, TLC method was used to test the extract's antioxidant, phenolic and polyphenol compounds. Gas Chromatography was applied to analyze the presence of chemicals in the plant extract. This study found that the extract of henna leaves contained antioxidants, phenolic, and polyphenol compounds. There are four chemicals found in the plant sample that show antibacterial, antifungal and antioxidant properties. This study proves that Henna leaves are composed of dye and other valuable compounds that could benefit the body. Hence, the application for this leave could be extended not only for dye purposes but also for others.

1 Introduction

Lawsonia inermis, commonly known as a henna tree, is originally from Northern Africa and Northern Australia but can also be found in Asia. It grows in tropical and subtropical regions. The Egyptians were among the earliest to use the henna plant. The leaves were used to colour the fingernails of mummies, and fragrant flowers were used to produce perfume. Henna is a medicinal plant widely used in medicine and ritual practices among the people of North Africa. In the therapeutic application, the henna leaves were mixed with Struthium

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(*Saponaria officinalis* L.) before being applied to the hair as a hair dye. Lawsone is the main ingredient in the henna plant. It is also known as hennotannic acid. Lawsone is a staining property or dominant dye produced by Henna. Lawsone contains a red-orange pigment [1]. Henna leaves crushed in an acid medium and rubbed on the skin cause lawsone molecules to migrate from the henna paste, traverse the outmost layer of the hair and colour the hair [2]. Crushed Henna in an acid medium is known as an extraction process.

Plant extraction is the process of separating active plant ingredients or secondary metabolites, commonly using a solvent. Methods of the extraction process started with selecting the best quality plant, collecting, and determining plant material [3,4]. Extraction is a crucial process to obtain bioactive compounds such as polyphenols, which contain antimicrobial properties [5]. Plant extracts are used as vitamin supplements and preserving agents.

Maceration is the process of extracting raw material in powder form from roots, tree trunks, and leaves by using a solvent [6, 7]. The ingredients are immersed in the solvent for a few days. It is also an affordable method to isolate bioactive compounds from plant-based material. Ethanol and acetone solvents are appropriate solvents used for maceration extraction [8]. The extraction process is conducted at room temperature. According to Mohammad Azmin et al. [4]; and Ashnagar and Shiri [9], maceration is an immersion process with or without heat. This maceration process can take 12 hours and up to three days. Based on the research paper [10], this experiment uses 80 g of henna powder dissolved in 4000 ml of distilled water. The maceration process uses a temperature of 75°C while using a magnetic stirrer. After one hour, the colour of the solution turns brown. The sample was purified using filter paper. The crude was discarded, and the brownish liquid was obtained for about 3400 ml. Other findings used maceration extraction by shaker and kept at room temperature. In this procedure, 96% of ethanol was added to the flask where the solvent colour change occurred at the minute of 150 [11]. The remaining crude plant sample turned sage green. The flask was covered with aluminium foil. The obtained yield is 10 ml. Mahkam et al. [12] utilized ethanolic extraction. In their study, 20 g of henna leaves and 100 ml of 70% ethanol were used using the maceration method for two hours. The study obtained 50 ml of the solution from henna leaves in light orange pigment. Although all three experiments use the maceration method, the total yield differs due to different heat applied [12].

The plant contains the active compound, where each plant composes of different bioactive compounds. The active ingredients in the plant include alkaloids, anthocyanins, and flavonoids. The active compound could be detected by the colour appearance of the band during tests like Thin Layer Chromatography TLC The disadvantage of this chromatography method is that it can only show the presence of compounds with different colour bands. Thin layer chromatography (TLC) aims to determine the compound in the plant extract. TLC is a popular analytical technique due to its ease of use, low cost, high sensitivity, and rapidity of separation. TLC aims to achieve well-separated spots. Yellow alkaloids were identified on the TLC plate. Terpenoids appear reddish-brown on the TLC plate. Tannins are found in plant samples in brownish-green and blue-black hues. Wagini et al. [13] tested the henna extract using the TLC method. Firstly, the henna powder was sprayed with n-hexane solution on a silica plate gel. The spot spread on the silica gel plate. The plate was then observed under 254 nm U.V. light. The travel distance of spots was measured. Rf values obtained are 0.22 (flavonoids), 0.33, and 0.39 (alkaloids) [13]. Mikhaeil et al. [14] used a G60 silica gel plate to test the bioactive compound on the henna extract. The plate was dotted with an extracted plant mixture. The silica plate was placed in the beaker containing ethanol. Silica plates were sprayed with hexane, and an orange spot appeared on the plate. The active compound has been found. The travelled distances were recorded as 0.20, 0.31, and 0.38, identified as flavonoids. The flavonoid's colour in the TLC method appears yellow. Based on other research, a TLC test on henna leaves was conducted using ethanol and sprayed with n-hexane.

A very pale yellow was obtained on the silica plate. The rf values recorded were 0.21, 0.25 and 0.31, known as alkaloids [12]. The colour of alkaloids is yellow and orange. All three reported experiments were conducted using the same method but with different equipment and materials. Therefore, the travel distance and mobile phase recorded are varied.

Therefore, this study aims to extract henna leaves using the freeze-dried method before testing the phytochemicals in the extract. In the literature search, previous studies use other extraction techniques, while this study focuses on the freeze-dried extraction method. This study also seeks to analyze the presence of chemicals in the plant extract. The Thin Layer Chromatography, TLC method was used to test the extract's antioxidant, phenolic and polyphenol compounds. Gas Chromatography was applied to analyze the presence of chemicals in the plant extract.

2 Methodology

2.1 Sample preparation

The Henna leaves were collected in Lakota, Jeli Kelantan, Malaysia. The henna leaves were selected, picked, and cleaned under running tap water to remove dust and dirt from the surface of the leaves. The initial weight of henna leaves was recorded.

2.2 Drying and grinding of plant sample

Henna leaves were dried for 17 hours at a temperature of 60 °C using a Biochef ST-00 dehydrator machine at 220 v, 50 Hz, and 5850 w. This process was conducted until the weight of the dried henna leaves reached a constant weight. The henna leaves were ground and finely powdered with a Philips NL9206AD grinder and continued with the sieving process using Retsch Vibratory Sieve Shaker AS 200 Basic for 20 minutes with an amplitude of 85. The leaves were sieved with 250 µm and 60 mesh size sieves to obtain the finest henna powders. This vibratory sieve shaker sieved the plant sample in throwing motion with angular momentum suitable for wet and dry sieving. The dried henna leaves were packed and sealed in polyethene bags before storing them in a chiller for further use.

2.3 Plant moisture content

Moisture content is crucial to plants as it affects the texture, weight, appearance, and crop yields. The moisture content of henna leaves was computed using Equation 1.

$$Moisture\ content\ = \frac{(Wet\ weight - weight\ after\ drying)}{Wet\ weight} \times 100\% \quad (1)$$

2.4 Extraction method for *Lawsonia inermis*

Henna samples were extracted with 60 ml of distilled water. The weight of the henna powder used is 0.5 g. Henna leave powder was extracted with the maceration method by using a Stuart US 152 hot plate. The extraction method was conducted at 70 °C for 80 minutes. The extracted samples were filtered with Whatman Qualitative Filter Papers 101. The filtrate was collected and stored in a Panasonic Ultra Low-Temperature MDF-U55V freezer at -80 °C overnight [15]. The samples were stored in CoolSafe 4-15L Freeze Dryer ScanVac LaboGene

for three days, where the initial weight of this freeze-dried sample was 50.76 g. The freeze-dried method has been used to preserve the volatile compound [16].

2.5 Phytochemical screening

Phytochemical screening is an essential step in detecting the appearance of phytochemicals in plant extract. Therefore, this study focuses on detecting active compounds in the extracted sample of Henna using Thin Layer Chromatography (TLC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Gas Chromatography tests.

2.5.1 Thin Layer Chromatography, TLC

According to Henna et al. [17], the Thin Layer Chromatography (TLC) test was performed using 25 ml of ethyl acetate and 25 ml of hexane liquids. A 1 cm horizontal line was drawn at the top and bottom of the plate. A henna extract sample was analyzed. On each plate, each sample was replicated three times. The experiment was conducted in a chamber, with the plate perpendicular to the solvent. Aluminium foils were covered over the beakers, and the solvent front was allowed to rise to the top line. After drying, the plate was examined under U.V. light, as shown in Figure 1 (a), while the compound travel phase is in Figure 1 (b).

2.5.2 1,1-diphenyl-2-picrylhydrazyl, DPPH

New DPPH (1,1-diphenyl-2-picrylhydrazyl) was made by dissolving 2.4 mg of DPPH in 100 ml of 80% aqueous methanol added with 20 ml of distilled water and filled in a dark reagent bottle. The identical plate and solution were used for the DPPH. test, the same as the TLC method, except the samples were sprayed with a DPPH reagent.

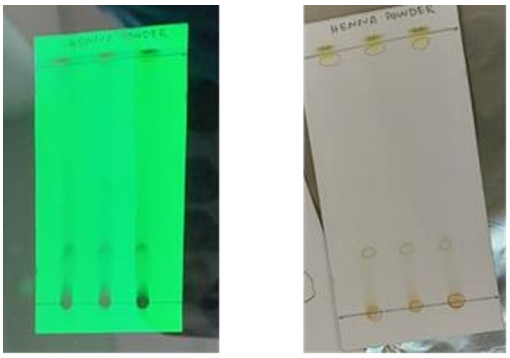

Fig. 1. (a) Henna sample was observed under U.V. light and (b) compound travel phase

2.5.3 Chromatography-Mass Spectrometry (GC-MS)

According to Sharma & Goel [18], the GC-MS test was conducted using 15 g of henna powder soaked with 250 ml of methanol solvent overnight. The sample was filtered with Whatman No. 1 and stored in a vial. GC-MS analysis was performed using (Agilent Technologies 7683). The fused silica was filled with HP-MS (5% of Phenyl-methylsulphoxane). The dimension is 30 m x 250 µm. The oven was heated to a temperature of 50°C with an increase of 3°C every minute. The maximum temperature for this oven is

240°C. Helium gas (99.999%) was transferred at a constant flow rate of 1ml/min. The 70 eV energy was applied for the electron ionization of molecules. This analysis has taken a total running time of 90 minutes.

3 Results and Discussion

3.1 Plant Moisture Content

The initial weight of henna leaves is 246.60 g. After being put into a dehydrator for 17 hours at 60°C, the original weight of the leaves decreased to 79.19 g. The calculated moisture content of henna leaves is 67.89 %. You et al. [19] found that the moisture content of tea leaves is in the range of 0 % - 50 %. The moisture content of these tea leaves was obtained through the oven-drying method at 105 °C for three to five minutes and was repeated until the leaf weight was constant [19]. The moisture content of henna leaves is higher than tea leaves because tea leaves use a high temperature during the drying process of samples compared to Henna at 60 ºC maximum temperature.

Meanwhile, the moisture content of banana leaves was recorded at 40 % - 82 % [20] as the leaves are more sensitive to the environment and absorb moisture more quickly because the trees are a type of plant fibre. The study dried the banana leaves using microwave oven drying. The moisture content is crucial to eliminate the possibility of an incorrect evaluation of the items' return loss performance [20].

The moisture content of pandan leaves that were dried using a cabinet dryer for six hours at 40 °C is 75.18 % - 80.91 % [21]. As a comparison, the pandan leaves' size is wider while henna leaves are oval, so the moisture content of pandan leaves is higher than that of the henna leaves. The leaf surface affects the drying rate and moisture content [21].

Kendall et al. [22] dried kenaf leaves using vacuum drying at 70 °C to avoid losing the main compound. Kenaf leaves are placed in a vacuum drying at 60 °C for three hours and at a pressure of 1.3 kPa. However, this drying method has reduced oxidation but high energy consumption compared to the dehydrator drying method, which is inexpensive and easy to handle. The moisture content of kenaf leaves was 7.30 %, much lower than that of henna leaves. Kenaf leaves have low moisture content because it is known as a cellulose fibres plant source. This plant does not carry and absorb water as the fibres are formed from damaged leaves.

Drying is essential to remove moisture from the sample or raw material. It affects the shelf life of the products, prevents microbial growth, and reduces the cost of packaging [23]. According to Kendall et al. [22], using the dehydrator machine method is very effective compared to the sun drying method. Dehydrator drying is highly recommended because it is cheap and suitable for small and large-scale drying processes. Dehydrator drying with thermostatic controls is very easy to operate. The appropriate drying method preserves samples by removing moisture content from samples to avoid spoilage or bacteria growth [24]. Adequate air circulation helps to carry off the moisture without affecting the main compound. In addition, using a dehydrator saves costs and speeds up the drying process quickly [25]. Heat sources and water flow help reduce the water content in henna leaves. However, the sun drying method takes a few days to dry the raw materials because the temperature cannot be controlled, and the leaves tend to be overheated at some point [15]. The night breeze air adds moisture content to the sample. A temperature of 30° C is the minimum temperature for sun-drying and requires hot, dry, and breezy days. The drying method is also unhygienic because it is exposed to insects, soils, and other contaminants. Therefore, the drying process does not happen ideally because sun drying requires ample space, and the possibility of losing crops is high due to weather changes [26].

3.2 Extraction Method for *Lawsonia inermis*

The initial weight of henna leaves is 50.76 g, while the weight of the Henna after freeze-dried is 17.29 g. The optimal extract was stored in a freezer using a Panasonic Ultra-Low Temperature Freezer at -80 °C for a day. Extracts were freeze-dried with an ice condenser temperature of -54 °C actual pressure of 651 hPa for three days.

According to Kashaninejad et al. [27], the recorded initial weight of olive leaves is 38.66 g, and after weight is 7.35 g when freeze-dried at 1.5·10−4 bar two days. This leaf is extracted with 80% ethanolic aqueous using a conventional solvent extraction method. Moringa leaves were extracted using a liquid-solid extraction method using 50% of aqueous ethanol. Ethanol was removed with a rotary evaporator. The samples were kept in the freezer - at 80 °C minimum for two hours, then removed into freeze-dried and frozen with liquid nitrogen at 196 °C for 48 hours. The recorded initial weight of the Moringa leaves sample is 29.5 g; after the freeze, the extract is 8.48 g [28]. Mulberry leaves were extracted with 70% ethanol by the Soxhlet extraction method to remove the chemicals. The sample was frozen at -20 °C for one day. Lyophilizer was used to dry the pieces at 49 °C. The final weight of the piece is 3 g [29]. The results from the three freeze-dried plants vary due to temperature, the volume of solvent used, the duration of time, and the extraction method of each plant sample [4,7]. Thus, this study found the amount of henna extract in the acceptable range, almost similar to the previous research.

3.3 Phytochemicals Screening

3.3.1 Thin Layer Chromatography, TLC

The recorded retention factors (Rf) for henna samples are 0.24, 0.23, and 0.21. Throughout the TLC. test, the active compound in henna leaves is detected. According to UH III [30], the henna powder was soaked in chloroform for 60 minutes. The filtrates were concentrated with 10% w/v. The henna sample is dripped on a silica plate. Chloroform and methanol are used for eluting as the mobile phase. Spots are identified as flavonoids due to their yellow colour [31]. The spots were identified under shortwave and longwave. Using silica gel, the lawsone compound was found in Henna hair dye paste on a TLC. test. The compound colour is red to orange. Lawsone is a natural compound found in henna leaves, and the mixture has been extracted with the maceration method. This natural compound is widely applied in cosmetics and traditional medicine.

3.3.2 1,1-diphenyl-2-picrylhydrazyl, DPPH

DPPH test is used to determine the presence of antioxidants in henna leaves. Antioxidants are detected in henna leaves. A blue and purple colour occurred after the TLC was sprayed with the mixture of DPPH Antioxidant provides a shield and acts as a treating agent. DPPH colour depends on the concentration of the sample solution [32].

3.3.3 Chromatography-Mass Spectrometry (GC-MS)

According to Mengoni et al. [33], 72 compounds have been identified in henna leaves using the GC-MS method. Pentadecanoic acid, 13-methyl-, methyl ester, has been identified and contains antibacterial and antifungal properties. Based on Udin et al. [31], n-hexadecanoic acid is found in 51 compounds of the henna plant. Antioxidants enhance hair growth and restore the collagen in the hair tissue cells. Vitamin E was also found by Dev, De and Khan

[34] in henna leaves to function as a protective lipid layer and provide a healthy scalp. It contains high antioxidants, which prevent breast cancer. Acetamide has antifungal properties.

Table 1. The compounds found in Henna leaves analyzed with GC-MS

No.	Compound name	Biological Activities
1	Pentadecanoic acid, 13-methyl-, methyl ester	antibacterial and antifungal
2	n-Hexadecanoic acid	Antioxidants
3	Vitamin E	Antioxidants, preservative agents and reduce breast cancer
4	acetamide	Antifungal

4 Conclusion

The present study reveals that the henna leaves contain beneficial compounds from phytochemical screening and chemical analysis. The compound could benefit skin and hair not only as a dye. The Henna contains antioxidant, phenolic and polyphenols from TLC. method analysis while showing antibacterial, antifungal and antioxidant from the chemical analysis. Therefore, Henna could be utilized for other purposes, not only for dye. The study could be extended to find the percentage of each compound found in the extract so that the suitable properties of the intended purpose could be predicted.

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