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Extraction of Phytochemicals in *Morinda citrifolia L.* Leaves by Using Different Polarity Solvents

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Abstract: *Morinda citrifolia L.* is known to have anti-inflammatory, antibacterial, antifungal, and antioxidant benefits due to its many phytochemical compounds present. Therefore, this research was conducted to determine the best solvent for the extraction of phytochemicals in *M. citrifolia* leaves by comparing three different solvents. The leaves powder was macerated respectively in three different polarity solvents which were dichloromethane, ethyl acetate, and n-hexane for 3 days. Dichloromethane had the highest percentage yield of extract (75.00%), followed by ethyl acetate (18.89%) and n-hexane with the lowest percentage yield (5.06%). This research found ethyl acetate was the best solvent for the extraction of phytochemicals in *M. citrifolia leaves* compared to dichloromethane which yield 75% of extract due to its ability to dissolve a wide range of compounds, both polar and nonpolar compounds present in the plant material while dichloromethane only dissolve polar compound.

Keywords: *Morinda Citrifolia L.*, Leaves, Maceration, Dichloromethane, Ethyl Acetate, N-Hexane

1. Introduction

Morinda citrifolia L. (Rubiaceae) is commonly as noni, is indigenous to tropical regions, including French Polynesia, Tonga, Hawaii, Australia, and other Pacific islands [1]. *M. citrifolia* is also referred to as *M. bracteata*, *M. litoralis*, Indian mulberry, Bengkudu, and Mengkudu. Based on historical, taxonomic, and regional circumstances, *Morinda citrifolia* has been given several names. Through organisations such as the International Plant Names Index (IPNI) and the International Code of Nomenclature for Algae, Fungi,

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and Plants (ICNafp), the scientific community frequently attempts to standardise and explain plant names. These organisations seek to provide a consistent and globally acknowledged standard for naming and classifying plants. The leaves appear to be the plant's primarily cultural use, as a topical wound recovery treatment. *M. citrifolia* leaf extracts have been used for centuries to treat or prevent a variety of chronic diseases, including reactive oxygen species (ROS) injury, diabetes, hypertension, and malaria. There are flavonoids, proteins, saponin, and tannins in noni leaves [2]. *Morinda citrifolia L. Morinda citrifolia L.* have anti-inflammatory, anti-bacterial, anti-fungal, and antioxidant properties, and have been shown to improve digestion. *M. citrifolia* is gaining popularity as a food additive, dietary supplement, and natural health booster. In addition, *M. citrifolia* leaves can initiate wound-healing activity. The leaves of *M. citrifolia* are used as topical medications to treat and alleviate discomfort in areas of damaged skin [3].

Due to its beneficial health compounds, Nayak et al. (2009) [3] conducted a phytochemical screening of *M. citrifolia* root extracts, detecting important bioactive components such as phenolics, flavonoids, tannins, steroids, and triterpenes using hexane, ethyl acetate, and dichloromethane. The different polarity of these solvents extract of noni leaves possesses wound healing activity and has been shown to be safe in acute, sub-acute and sub-chronic oral toxicity tests on mice [3].

The maceration method is an old technique in which the entire or selected parts of a vegetable sample are kept in contact with a specified solvent for a period that can range from a few hours to days, at room or higher temperatures. Double maceration is the maceration process for concentrated preparations or the multiple maceration process [4]. The leaves were macerated twice in that process using three different solvents divided into two parts. Each maceration was carried out at a different volume. Due to a significant quantity of active principle that may be left behind in the first pressing of the marc, repeated maceration may be more efficient than single maceration. In circumstances where active elements are more beneficial, repeated maceration is more efficient. The purpose of this study is to extract the phytochemicals present in *M. citrifolia* leaves and compare the effects of various extraction solvents based on their polarity.

2. Materials and Methods

2.1 Materials Specifications

M. citrifolia or noni leaves were collected from Marang, Terengganu. The different types of solvents used are dichloromethane (100%) (polar), ethyl acetate (100%) (moderately polar), n-hexane (95%) (nonpolar). Distilled water was used to produce saponins, and ferric chloride solution produced tannins, phenolics, and flavonoids. To produce alkaloids, Wagner's reagent of brand R&M which were produced in Selangor Darul Ehsan was used followed by chloroform and concentrated sulfuric acid to form terpenoids.

2.2 Phytochemical Screening Methods

Extraction of phytochemicals was observed by using a double maceration extraction method where the *M. citrifolia* leaves were suspended in each solvent for 3 days [6]. First, the *M. citrifolia* leaves were collected and washed with water. *M. citrifolia* leaves were dried in the oven for 24 hours at 60°C and powdered in an electrical blender [5]. Then, 24.84 g of the powder was suspended in 400 ml of dichloromethane, ethyl acetate and n-hexane for 3 days at room temperature, individually [6]. This mixture was filtered using Buchner funnel. The extraction was suspended again with 100 ml for each solvent and then filtered after 3 days. Phytochemical screening was obtained by using a few tests.

2.3 Phytochemical Screening Methods

Saponins

20 mL of distilled water was added to 1 mL of *M. citrifolia* extract and shaken for 15 minutes in a graduated test tube. Formation of layer of thick foam 1 cm indicate the presences of saponins [3].

Tannins

In a volumetric flask, 5g of ferric chloride was diluted with 100 mL of water. 1 mL of the *M. citrifolia* extract received 3–4 drops of ferric chloride solution. A brownish-green or blue-black color indicated the presence of tannins.

Phenolics

In a volumetric flask, 5g of ferric chloride was diluted in 100 mL. Three to four drops of ferric chloride solution were added to 1 mL of the *M. citrifolia* extract. Phenols were present as shown by the production of blue-black color.

Flavonoids

To the 1 mL *M. citrifolia* extract solution, add a few drops of ferric chloride solution. The presence of flavonoids was indicated by the appearance of an intense green color.

Alkaloids

2 gm of iodine and 6 gm of potassium iodide were dissolved in 100 mL of distilled water to make Wagner's reagent. A few drops of Wagner's reagent were added to 1 mL of *M. citrifolia* extract, and the formation of a reddish-brown precipitate by reaction indicated the presence of alkaloids.

Terpenoids

To *M. citrifolia* extract solutions, carefully added 0.5 mL of chloroform and 1 mL of concentration sulphuric acid to form a layer. The presence of terpenoids was indicated by the formation of a reddish-brown coloration of the interface.

2.3 Percentage yields calculation

The weight of the noni leaves before and after extract with 3 different types of solvent was determined. The percentage yield of weight for noni leaves is calculated using Eq. 1.

$$\square = \frac{[\square_{\square} - \square_{\square}]}{\square_{\square}} \times 100\% \qquad \text{Eq. 1}$$

where R is the percentage yield (%), C_i is the initial value weight of the plants and C_f is the final value weight of the plants.

3. Results and Discussion

3.1 Extraction of *M. citrifolia* leaves

Table 1: Percentage Yield of Extraction

Solvent	Percentage Yield (%)
Dichloromethane	75.00
Ethyl acetate	18.89
n-Hexane	5.06

Three different polarity solvents; dichloromethane, n-hexane, and ethyl acetate were used for an extraction technique. Each solvent has a different ability to extract chemicals from *M. citrifoliae* (Table 1). The yields obtained from dichloromethane, ethyl acetate and n-hexane solvents are 75.00%, 18.89% and 5.06% respectively. Thani, et al. (2010) found that the yield of dichloromethane was 0.79%. However, due to the extraction method used in this study, a double maceration method, the yield was 75% [7]. Double maceration increases extraction efficiency which increases the yield of extraction. Repeated maceration may be more effective than a single maceration because significant amounts of the active principle may be lost during the first pressing of the marc [8]. Dichloromethane has a higher polarity than ethyl acetate, so dichloromethane yields a greater % of *M. citrifolia* leaf extract. The solvents dichloromethane and ethyl acetate can extract both polar and nonpolar substances while n-hexane can only extract nonpolar compound.

Based on previous research, all *M. citrifolia* root extracts contained low-polar secondary metabolites such as triterpenes and steroids, according to the results of the phytochemical investigation. Hexane extract contains the highest concentration due to the low polarity of these compounds and their high solubility in non-polar organic solvents [9]. Stitcher (2008) claimed nonpolar solvents (such as hexane) yield more lipophilic compounds, while polar solvents (such as alcohol) produce extracts containing a wide range of polar and nonpolar compounds, which increases extraction yields [10]. In summary, the polarity of the extracting solvents had a significant impact on extraction yield and phytochemical content. When compared to non-polar solvents, extraction in highly polar solvents resulted in a higher extract yield but a lower phenolic and flavonoid concentration. A combination of polar and nonpolar solvents can be used to improve the extraction efficiency of phytochemicals with high antioxidant properties from noni leaves

3.2 Phytochemical Screening

Table 2: Phytochemical Screening

Phytochemical	Observation		
	Dichloromethane	Ethyl acetate	n-Hexane
Flavonoid	+	+	+
Saponin	-	+	+
Terpenoid	-	-	-
Phenolic	-	-	-

Tannin	-	+	+
Alkaloid	-	-	-

Notes: The symbols of '+' and '-' represent presence and absence, respectively.

Based on Table 2, the flavonoid is mainly found in *M. citrifolia* leaves, indicating it is a major antioxidant compound. Flavonoids, saponins, and tannins are the active compounds found in *M. citrifolia* leaves extract, and they are denoted by the symbol (+) in Table 2. All the extracts contained flavonoid compounds as their primary bioactive components, but the dichloromethane and ethyl acetate extracts contained more than the hexane extract. This can be attributed to the presence of hydroxyl groups on the aromatic rings of flavonoid compounds, which gives them a high degree of polarity [9]. Several studies have found that n-hexane extract does not contain flavonoids [11]. In spite of this, flavonoids were found to be present in the current study. This is usually the result of the extraction process, which can lose heat and degrade the quality. The main bioactive components of *M. citrifolia* are flavonoid compounds, which are primarily detected in the leaves when they are extracted using dichloromethane, ethyl acetate, and n-hexane. Tannins and saponins in *M. citrifolia* are only detectable in ethyl acetate and n-hexane extracts, but not in dichloromethane extract. It reveals that for terpenoid, phenolic, and alkaloid compounds, dichloromethane, ethyl acetate, and n-hexane extract are not present in these plants. Based on the previous study, ethyl acetate extract had the highest tannins content [12] as well as our findings.

Ethyl acetate is a moderately polar solvent able to dissolve a wide range of polar and nonpolar compounds in plant materials. However, dichloromethane extract has a lower detection of phytochemical screening compounds since dichloromethane is generally more effective at extracting nonpolar compounds such as lipids, hydrocarbons, and certain nonpolar terpenoids. Different geological locations give the plants different nutritional values, which is primarily explained by the difference in geological locations [11]. Geological factors can have a significant impact on phytochemical extraction from plants because they affect the composition and properties of the soil, which affects plant growth and the accumulation of bioactive compounds. In addition, double maceration increases dichloromethane concentration and reduces phytochemical detection. It is preferable to use the Soxhlet method to avoid the disadvantages of consuming too much solvent and their complexity [13][14].

4. Conclusion

It can be concluded that ethyl acetate is the optimal solvent for phytochemical extraction from *M. citrifolia* leaves due to its ability to dissolve both polar and nonpolar compounds present in the plant material. Further investigation is necessary to determine the efficacy of the solvent-based method in relation to polarity for the identification of active constituents derived from this particular plant. The utilisation of double maceration extraction is deemed more efficient due to the potential retention of a substantial amount of the active ingredient, which may be left behind during the initial pressing of the marc.

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