



## Extraction of Neem Balm from *Azadirachta Indica* Leaves using Soxhlet Method

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KEYWORDS	ABSTRACT
Silver nanoparticle; Neem leaves; Neem balm; Stability; Extraction.	Neem ( <i>Azadirachta Indica</i> ) may be the most useful traditional medicinal herb in India. Each component of the neem tree has some medical value and can therefore be used commercially. It is today regarded as a valuable source of distinctive natural materials for the creation of both industrial items and medications to treat a variety of ailments. The objectives of this research were to extract neem leaves by using a distillation method, precisely the Soxhlet method, and to analyze the balm stability. The three types of balm were prepared; empty balm, neem balm, and neem balm with silver nanoparticles. In the Soxhlet extraction method, the optimal operational state for recovering oil was an extraction period of 24 hrs, 300 ml of ethanol solvent at a temperature of 79 °C and 60 g of powdered neem leaves. The maximum percentage of yield obtained was 35.0 %. The confirmation of neem oil extract was done by UV-Vis Spectroscopy. The balms' thermal stability was evaluated at room temperature (25 °C) and 45°C in an oven. After three months of study, all of the balms; empty, neem, and neem with silver nanoparticles showed stability at 25 °C without undergoing any physical changes. Otherwise, after two months of stability testing at 45 °C, neem balm and neem balm with silver nanoparticles showed signs of sedimentation.

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### 1.0 INTRODUCTION

*Azadirachta Indica*, also commonly known as the neem tree is one of the very few trees known in the Indian subcontinent. This tree, which is a member of the Meliaceae family, thrives in tropical and subtropical climates. Additionally, it has been noted that this tree might endure arid situations. *Azadirachta Indica* has numerous beneficial substances that are used as insecticides and could be utilized to defend stored seeds against insects. However, in addition to potential health benefits such as blood sugar-lowering abilities, and anti-parasitic, anti-inflammatory, anti-ulcer, and hepatoprotective actions, harmful consequences are also mentioned [1–2].

The usage of herbal remedies in place of synthetic or chemical medications has grown in recent years as a result of the less severe side effects associated with earlier antibiotics [3]. Herbs can be utilized directly as their active ingredients or as plant extracts. The majority of the world's inhabitants

also employ herbal remedies because of their potent antibacterial effects and advantages for basic healthcare [3–4].

Numerous investigations have been conducted to investigate the antibacterial properties of neem extract. In the year 2023, Hashim et al. [5] reported the lotion with diluted neem in CO and aloe vera had the best result against *Staphylococcus aureus* sp. and *Pseudomonas aeruginosa* sp. with an inhibition diameter zone of 19.3 mm and 9.6 mm, respectively as shown in Figure 1. The addition of neem leaf extract to the seaweed biopolymer matrix was reported by Uthaya Kumar et al. in the year 2020 [6]. The most effective way to improve the properties of a seaweed-based film was to add 5 % w/w neem leaf extract and provide a 2.5 kGy gamma irradiation treatment. The film's antibacterial qualities were strengthened against microorganisms. In a different investigation, the Minimum Inhibitory Concentration (MIC) test was used to determine the antibacterial activity of neem extracts combined with ethanol and water against *M.roseus*, *E. coli*, *B.licheniformis*, *M.luteus*, and *S. aureus* [4]. *Salmonella*, *pseudomonas*, and *E. Coli* were all susceptible to the significant antibacterial activity of methanolic neem extract, according to another study [7].

Because of their distinct physical and chemical characteristics, silver nanoparticles (AgNPs) are being employed more and more in a variety of industries, including food, medicine, consumer goods, and healthcare. It is known to have potent inhibitory and bactericidal effects in addition to its significant significance in the fields of high-sensitivity biomolecular detection, catalysis, biosensors, and medicine. It also has anti-fungal, anti-inflammatory, and anti-angiogenesis actions. Researchers discovered that a cream formulation of AgNPs, which were biosynthesized using an extract from *Withania somnifera*, had antibacterial activity [8]. AgNPs and AgNO<sub>3</sub> cream formulations were made, and their antibacterial efficacy against plant pathogens (*Agrobacterium tumefaciens*) and human pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, and *Candida albicans*) were examined. The outcomes demonstrated that AgNP creams had far stronger antibacterial efficacy against the pathogens under test.

Oil-containing materials, such as neem seeds, kernels, fruits, flowers, etc., are treated with a low-boiling-point solvent in the solvent extraction process [9]. Because it is inexpensive, nonpolar, nonreactive, and highly soluble in lipids, glycerides, and hydrocarbons at low temperatures, hexane is the most widely used solvent. Because the solvent extraction process yields transparent oil with a high yield (92.3%–99.1%) and three times more azadirachtin content than mechanical extraction, it is chosen. One of the 180 dangerous air pollutants specified in the US EPA's Clean Air Act is hexane. Therefore, ethanol has gained attention as a solvent for oil extraction since it is an effective, safe, and ecologically acceptable alternative with non-flammable and less toxic qualities [10–11].

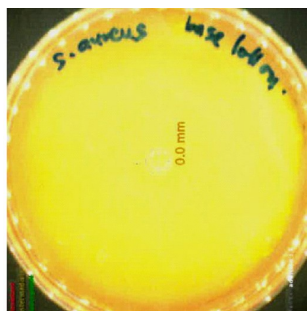
The most effective solvent needs to be looked at to maximize the extract yield. In a prior study [12], the Soxhlet and immersion extraction methods were compared to see which produced a higher concentration of neem extract. Several solvents, including methanol, ethanol, ethyl acetate, and hexane, were employed as an extraction medium. The Soxhlet method's 21.5 % yield percentage and the immersion method's maximum yield percentage of 22.0 % both showed that ethanol enhanced extract output and was obtained at 90 °C of extraction temperature. In another study [13], Subramanian et al. suggested the neem oil Soxhlet extraction by using hexane and ethanol as non-polar and polar solvents. They found that the highest oil and azadirachtin yields were obtained at 6 hrs extraction time using a 50:50 solvent mixture for both neem leaves and seeds.

Neem oil can also be extracted using supercritical fluid extraction (SFE) [14–16], and mechanical pressing [17–19]. The mechanical crushing of the seed at a regulated or cold temperature is the procedure used to extract neem oil. The most used extraction method is mechanical extraction since it is affordable, convenient, and solvent-free. Approximately 82% of neem oil is extracted using this approach. However, because of the low azadirachtin concentration, large amounts of water, and metal that cause the oil to become murky, the oil produced using this process is of low quality and low market value [20]. Oil-containing materials, such as neem seeds, kernels, fruits, flowers, etc., are handled with a low-boiling solvent in the solvent extraction process. Because it is so effective at extracting oils with high purity and no solvent or metallic residue, the SFE method is gaining a lot of

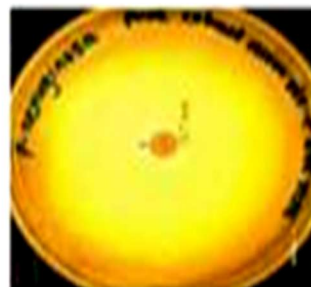
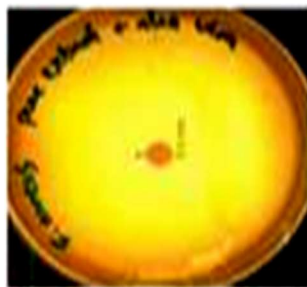
attention [21–22]. However, due to its high initial and ongoing costs, it is not widely utilized. Beyond the critical temperature of 31 °C and pressure of 74 bars, CO<sub>2</sub> is a liquid that is employed as the solvent in the supercritical fluid extraction process to extract neem oil and its active ingredients. SFE is eco-friendly, produces no waste, and doesn't require intense heating for oil extraction because CO<sub>2</sub> is a cheap, nontoxic, green, and non-hazardous solvent. Furthermore, because CO<sub>2</sub> is used to lower the danger of oxidative deterioration, SFE-derived oils have a longer shelf life. Solvent extraction is more cost-effective for extracting neem oil on a wide scale than solvent-flow ethanol since it requires less capital and operating costs.

Neem leaves have a long history of being a highly beneficial herb plant in both conventional and contemporary herbal medicine. To discover the ideal circumstances for neem oil extraction and the efficacy of neem balm, research on this material is required. Hence, this research was carried out to determine the yield synthesized from Soxhlet distillation. The stability of the neem balm with different formulations was also examined.

a) Base lotion



b) Lotion with pure neem oil and aloe vera



c) Lotion with diluted neem oil in coconut oil

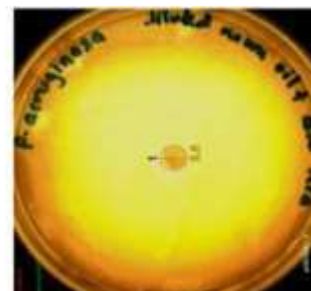


Figure 1: Bactericidal activity of base lotion and neem lotions with different bacteria analyses; *Staphylococcus aureus* sp. (left) and *Pseudomonas aeruginosa* sp. (right) [5]

## 2.0 METHODOLOGY

### 2.1 Materials and equipment

The neem leaves were obtained from the neem trees at University College TATI, Kemaman. The neem leaves were rinsed with water. They were then dried up in an oven, at 109 °C, for 28 hrs and were blended to powder using a blender for 1 minute. The temperature and time are suitable for the neem leaf drying process. Figure 2 shows the dried neem leaves.



Figure 2: Dried neem leaves

### 2.2 Extraction of Neem Oil

60 g of powdered neem leaves were weighed into the thimble, and 300 ml of 95% ethanol, the solvent, was added before putting the thimble into the Soxhlet apparatus and heating it. The setup was heated from 4hrs to 28 hrs, at a boiling point of ethanol, 80 °C after the thimble was fixed into the Soxhlet apparatus. From the reflux condenser, the gas fell into the sample in the thimble during the boiling of the solvent and condensation of the gas. The oil from the leaves was mixed and carried with the ethanol droplets. The liquid in the Soxhlet apparatus turned from a clear to a light-yellow tint. The final product was pure neem oil.

### 2.3 Neem Balm Formulation

60 g of shea butter, 50 g of cocoa butter, 20 g of castor oil, and 10 g of sweet almond oil were placed into a double boiler. The oils and butter were placed in a glass beaker which floated in a beaker of hot water at 80 °C. The point of the double boiler (Bain-marie) is to heat the oils slowly, evenly, and through an indirect heat source. Next, the oils were melted over medium heat at 80 °C until they were completely liquid. The bowl was stirred and placed in a freezer for five minutes until the oils thickened to the consistency of castor oil and became slightly opaque. The bowl was beaten with a whisk, adding 3.6 g essential oil, and 1.42 g of vitamin E. As for the neem balm, 5 g of neem oil was added and for the silver nanoparticles balm, 5 g of neem oil and 5 g silver nanoparticles were added.

### 2.4 Neem Analysis

The various molecule absorbs of ethanolic neem oil at the various particular wavelengths of the UV-vis area, 200 to 500 nm, were measured using the Shimadzu UV-1800 Ultraviolet-Visible Spectroscopy (UV-vis) equipment. Using a 200 nm/min scanning speed and 0.1 nm bandwidth, the absorption bands of each functional group that was present in the sample were identified.

Neem leaves were soaked, cleaned, and dried at 109 °C for 24 hours before the moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100\% \quad \dots \text{Eq. (1)}$$

Where  $W_1$  is the weight of neem leaves before drying (g),  $W_2$  is the weight of neem leaves after drying (g).

The yield % used to determine how much neem oil was extracted from 60 g of powdered neem leaves was as follows:

$$\text{Yield of oil (\%)} = \frac{\text{Mass of oil obtained(g)}}{\text{Mass of raw material used(g)}} \times 100\% \quad \dots \text{Eq. (2)}$$

In the thermal stability test, the balm samples were stored at room temperature and an oven at 45 °C for 3 months. The storage at 45 °C in three months of stability is equal to stability for two years at room temperature [23].

### 3.0 RESULTS AND DISCUSSION

The UV-vis spectrum of neem leaf extract is displayed in Figure 3. The simplest method to gain information of extract is this analysis. Three peaks were found, the first peak had an optical density of 0.269 at  $\lambda_{\text{max}} = 300$  nm, the second peak had an optical density of 0.249 at  $\lambda_{\text{max}} = 380$  nm, and the third peak had an optical density of 0.249 at  $\lambda_{\text{max}} = 390$  nm. This finding was supported by another study as reported in the year 2023 [24].

The extracted neem oil using 300 ml of 95% ethanol as a solvent at different time intervals was recorded in Figure 4. From this figure, the influence of time of neem oil recovered following the 28 hrs of Soxhlet extraction process. The first 4 hrs of the extraction produced almost no neem oil. Most of the neem oil is distilled in 12 hrs. Extending the extraction period resulted in a consistent in increasing the amount of neem oil being recovered. The optimum yield of neem oil was obtained after 24 hrs of extraction which is 35.0 % of yield. The extraction time was proven to affect oil recovery. Utilizing various solvent types and parameters, Soxhlet extraction and a simple distillation procedure were used to extract essential neem oil from neem seeds as reported by Tesfaye et al. by using hexane, methanol, and ethanol [25]. The greatest yield in Soxhlet extraction was 43.71%, derived from a combination of ethanol and hexane with volume proportions of 60:40%. The results of a basic distillation process showed that a maximum of 42.35% of neem oil could be generated by utilizing hexane. Particle size for all solvent types is 355  $\mu\text{m}$ , with extraction times ranging from one hour to three hours, and applied at both constant and variable temperatures. Hexane yielded more oil at a shorter extraction time than methanol and ethanol. Oil was not produced by ethanol in Soxhlet extraction after an hour of extraction. The quality and amount of neem oil can therefore be investigated by carefully determining variables like temperature, time, solvent type, and particle sizes.

Stability testing is done to make sure that, when stored under the right conditions, an improved product fulfills the desired criteria for physical, chemical, and microbiological quality as well as functionality and aesthetics. Stability test procedures typically follow the manufacturer's protocol, which calls for a year of analysis at room temperature and three months at 45 °C [23]. Predicting the stability of the cosmetic product over the course of its useful life and the compatibility of the formulation with the container's material are two methods of gathering information [26].

The stability testing is basically an experiment where a batch of the formula is made and exposed to different environmental conditions for a set period. These conditions, which vary in humidity and

temperature, are intended to simulate what would happen to the product over its lifetime. As a result, the following stability test technique should establish the test parameters that evaluate stability samples. Figure 5 shows the stability test of balms at room temperature. The three types of balms; empty balm, neem balm, and silver nanoparticle neem balm were prepared. All of them exhibit stability with no layer appearance or color changes. The neem sedimentation was not observed after three months of analysis. At 45 °C stability test, the difference observation was detected in Figure 6. After the first month, all of the balms showed no changes physically. After the second month, the neem balm exhibited a little sedimentation at the bottom of the container. Three months of analysis made the sedimentation appear more. Meanwhile, the neem balm with silver nanoparticle texture started to change as it darkened at the center, indicating the neem oil started forming sedimentation in the middle of the container after two months of observation. Most of the neem oil sediment appeared in the middle of the container after three months of study.

The neem balm can become more stable by making sure that all its ingredients are fully emulsified with each other to avoid sedimentation. The emulsifier for the balms in this study are the two types of butter which are cocoa butter and shea butter. From this study, it can be concluded that these two emulsifiers are not enough to emulsify the neem oil with other ingredients. So, to get the best neem balm, we need to add another emulsifier that is safe to become part of the balm [27]. Natural emulsifiers are the best option because they are safe for the skin. There are a lot of options for organic emulsifiers out there such as Xylance [28], Lecithin [29], and Olivem [30].

The European Cosmetic Toiletry and Perfumery Association (COLIPA) produced the guidelines on stability testing of cosmetic products [31]. The purpose of this paper is to lay out standards for forecasting and guaranteeing the stability of goods on the market. Its goal is to support cosmetic product makers in choosing and improving the right stability tests. While this guidance might be a useful beginning point, it is crucial that producers thoroughly assess new goods and technologies and, when necessary, modify their testing procedures to account for variations in product types and formulas. It is crucial to consider that items may change their qualities with age when creating a protocol for stability testing [32–33]. This means that testing properties other than those that would be assessed for initial release testing may be necessary for stability testing. The evaluation of the ensuing standards is provided as an illustration only; it is neither comprehensive nor minimal since the tests will vary based on the kind of product and packaging such as:

- Color, odor, and appearance
- Changes in the container
- pH
- Viscosity
- Weight changes
- Microbial tests demonstrating the ability of the products to prohibit microbial
- Growth during normal use and other specific test, if necessary,
- Analytical data about other parameters for specific product types

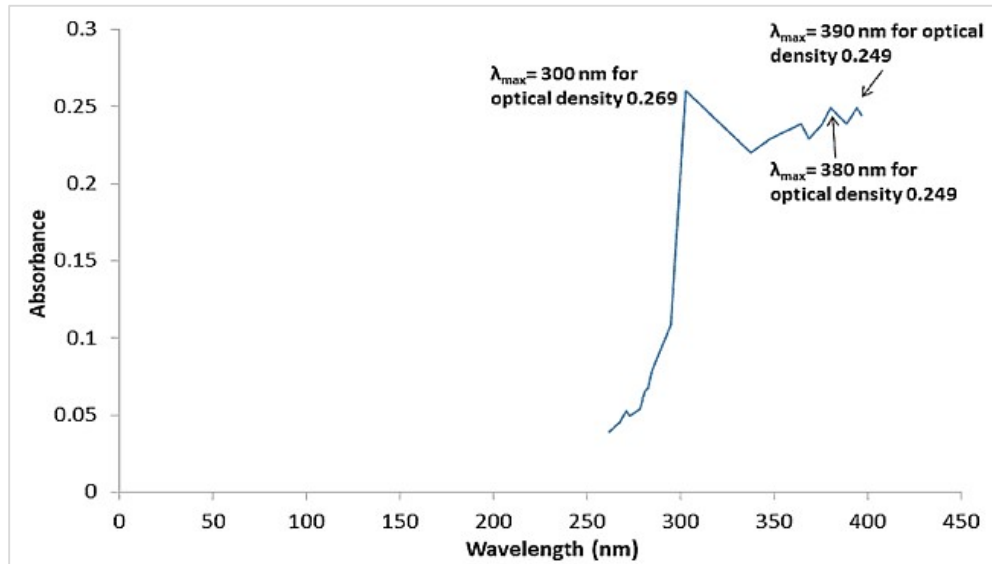


Figure 3: UV-Vis spectrum of neem oil by using ethanol extraction

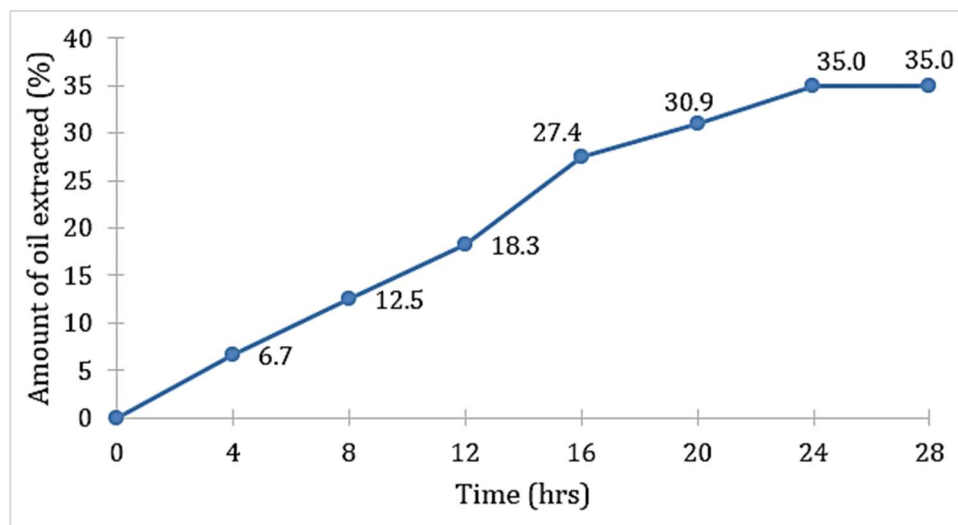


Figure 4: Yield percentage of neem oil after 28 hrs of extraction







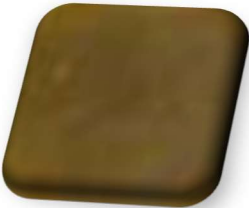




Figure 5. Stability test of balms at room temperature

Condition: (25 °C)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month
Balm			
	Balm was stable, no layer appeared, no color changed, and no sediment was observed	Balm was stable, no layer appeared, no color changed, and no sediment was observed	Balm was stable, no layer appeared, no color changed, and no sediment was observed
Neem balm			
	Balm was stable, no layer appeared, no color changed, and no sediment was observed	Balm was stable, no layer appeared, no color changed, and no sediment was observed	Balm was stable, no layer appeared, no color changed, and no sediment was observed
Neem balm with silver nanoparticle			
	Balm was stable, no layer appeared, no color changed, and no sediment was observed	Balm was stable, no layer appeared, no color changed, and no sediment was observed	Balm was stable, no layer appeared, no color changed, and no sediment was observed



Figure 6. Stability test of balms at 45 °C

Condition: (45 °C)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month
Balm			
	Balm was stable, no layer appeared, no color changed, and no sediment was observed	Balm was stable, no layer appeared, no color changed, and no sediment was observed	Balm was stable, no layer appeared, no color changed, and no sediment was observed
Neem balm			
	Balm was stable, no layer appeared, no color changed, and no sediment was observed	Balm was stable, no layer appeared, no color changed, however, some neem oil starts to form sediments	Balm texture was stable, but most of the neem oil became sediment
Neem balm with silver nanoparticle			
	Balm texture was stable, no color changed and no sediment was observed	Balm texture started to change as it darkened at the center, indicating the neem oil started forming sedimentation in the middle of the container	Most of the neem oil sediment appeared in the middle of the container

## 4.0 CONCLUSIONS

The neem oil was successfully extracted by using the Soxhlet extraction method and was confirmed by UV-Vis Spectroscopy. Maximum neem oil yield was obtained after 24 hrs of extraction in ethanol. At room temperature, all of the balms; empty balm, neem balm, and neem balm with silver nanoparticles showed stability without physical changes after three months of analysis. Otherwise, the neem balm and neem balm with silver nanoparticles exhibited the sediment appearance starting with two months of stability test at 45 °C.

## Author Contribution

M.R.A. Zakkir Muzammil: Conceptualization, methodology, investigation. H. Lili Shakirah: Investigation, supervision. Author: Methodology, visualization, writing, and editing.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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