TITLE:

EFFECT OF USING DIFFERENT TYPES OF SOLVENT IN THE EXTRACTION OF *AZADIRACHTA INDICA* (NEEM LEAVES)

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ABSTRACT

Azadirachta Indica is one of the greatest herbs and contains several bioactive compounds which are believed to possess several therapeutic properties such as antibacterial, antimicrobial and anti-inflammatory disease. These bioactive compounds can be obtained by using an optimum and appropriate extraction parameter. The extraction parameters include suitable drying methods, suitable solvents to extract polar or non-polar compounds, types of extraction technique and others. However, the comparative scientific study on the effect of using different extraction parameters towards the extraction yield and amount of extracted bioactive compound has been very limited. Therefore, this study aimed to determine the effect of using different types of extraction parameters focusing on different types of solvents towards the extraction yield and amount of extracted bioactive compound. The extraction process was implemented by using soxhlet technique by using Acetone, Ethyl-Acetate and Hexane as solvents. Then, the concentration of Gallic acid was determined by using High-Performance Liquid Chromatography (HPLC). The findings revealed that the highest extraction yield was shown by the extract from Ethyl Acetate with the value of 13.26%. While that gallic acid was present in every extract and that the highest amount of gallic acid shown by the extract from Hexane with the value of 63.833 ppm. This finding showed that extraction efficiency varied depending on the solvent utilized. The highest concentration of Gallic acid was found in the hexane extract. This study highlights that *Azadirachta Indica* or neem leaves are one of the useful plants that contain most bioactive compounds such as Gallic Acid and it could be optimally extracted by using Hexane.

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CHAPTER 1: BACKGROUND

1.1 Introduction

Azadirachta Indica is commonly known as Neem Tree. It is typically found in Tropical and Subtropical regions. Nowadays, *Azadirachta Indica* is commonly used in many healthcare products because it can be used as a cure to some diseases like Malaria fever, Gastrointestinal disorder and skin diseases. Every part from Neem tree is useful for example, the seeds and fruits from the tree can be used as Neem Oil, Neem leaves can be used as Diabetes treatment and can be used for the hair to nourish it and helps get rid of dandruff, the bark can be used to control fever, clean teeth, and treat loss appetite. Bio active compounds from *Azadirachta Indica* in Neem Plant have polar properties that can attract and repel each other. To extract the bio active compound in *Azadirachta Indica* we need to use polar solvent by using the extraction process.

1.2 Literature review

1.2.1 *AZADIRACHTA INDICA*

Azadirachta indica, known as neem (Indian subcontinent) is a part of the Meliaceae family. *Azadirachta indica* has long been used for both commercial and therapeutic purposes. The neem tree has medicinal qualities in all of its parts (Saxena et al. 2010). The plant's many parts, including the flowers, leaves, seeds, and bark, have been used as an insecticide, antibiotic, larvicidal, antimalarial, antioxidant, antibacterial, antiviral, and spermicidal as well as to treat both acute and chronic human ailments (Jeelani et al. 2022). It also has been used for thousands of years as a traditional treatment for a wide range of human illnesses. Neem has uses outside of agriculture as well. It is a broad-spectrum insecticide and fertilizer that is acknowledged globally. Research in dentistry, food safety, bacteriology, mycology, virology, and parasitology is now being done to learn more about the wide antibacterial activity of *Azadirachta Indica*(Marina and Merrell 2022).

Azadirachta indica initially sparked interest due to its potential as a non-toxic infection-control tool for agricultural application (Govindachari, 1992). Along with the uses of *Azadirachta Indica* in oncology, dentistry, dermatology, and endocrinology, modern medicine and infectious disease researchers have more recently begun to pay attention to the neem tree as a potential source for potential antimicrobials. Neem and neem-related products have the ability to target pathogens that are resistant to first-line antibiotics, bacteria that harm oral health and/or build challenging biofilms, fungal infections that endanger food sources, and viruses that have significant negative effects on human health. There are hundreds of different substances present in neem trees, such as phytochemicals, many of which have been discovered to be bioactive and have a variety of uses on their own. Some of the more prevalent phytochemicals found in the

neem tree, including azadirachtin, gallic acid, and nimbolide, have previously been identified as potential medications with a variety of biological functions. More than 300 distinct compounds have been found in the neem tree (Saleem et al., 2018; Braga et al., 2020; Nagini et al., 2021).

1.2.2 SOXHLET EXTRACTION

One of the most widely used methods for removing analytes from solid materials is called soxhlet extraction (Zygler et al.2012). It has been applied to a variety of samples, including soils, sediments, and plant and animal tissues. There are many different solvents that can be utilized, including dichloromethane (DCM), either in pure form or combined with acetone, hexane, or both (Boer et al. 2005). The main chamber of the Soxhlet extractor is filled with a porous thimble containing a solid sample. The extraction cycle is frequently repeated by refluxing the solvent through the thimble using a condenser and a siphon side arm. Soxhlet extraction is a reliable, time-tested method that allows for unattended extraction. But it necessitates a protracted extraction period and substantial solvent usage (Kim et al. 2012).

Soxhlet extraction is frequently used to extract beneficial bioactive chemicals from a variety of natural sources. A tiny amount of the dry material is placed in a thimble for this extraction and placed in a distillation flask with the solvent of interest. The thimble-holder solution is inhaled by a siphon once it reaches an overflow level, and the siphon then dumps the solution back into the distillation flask. The extracted solutes are introduced into the bulk liquid by this solution. The solvent returns to the solid bed of samples while the solute stays behind in the distillation flask. Up to complete extraction, the procedure is repeated (Saim et al. 1997).

1.3 Problem Statement

There are various *Azadirachta Indica*-based products that can be obtained from the local market including soap, personal care products and others. Its therapeutic activity was believed to be contributed by its bioactive compounds. However, the comparative scientific study on the extraction parameter towards the extraction yield and amount of Gallic acid has been very limited. Therefore, this study aims to identify the best solvent that gives the highest value of extraction yield and amount of Gallic acid using HPLC.

1.4 Objective

The objective of this study is to determine the best solvents in the extraction of *Azadirachta Indica* by measuring the highest percentage of extraction yield and highest amount of Gallic acid detected by HPLC