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Effects of different extraction solvents on phytochemical constituents of the leaf of *Moringa oleifera* Lamarck (Moringaceae)

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Abstract
Moringa oleifera, commonly known as the drumstick tree, is a widely utilized plant renowned for its rich nutritional content and numerous medicinal properties. The leaf of Moringa are particularly valued for their high concentrations of vitamins, minerals, and bioactive compounds, including flavonoids, phenolic acids, alkaloids, and saponins, which contribute to its antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. The extraction of these bioactive compounds is a crucial step in exploring the therapeutic potential of Moringa, and the choice of extraction solvent plays a significant role in determining the types and yields of phytochemicals obtained. This study aimed to investigate the effects of various extraction solvents on the phytochemical constituents of Moringa oleifera leaf. Using standard extraction techniques, the leaves were subjected to solvent-based extractions, and the resulting extracts were analyzed for their chemical composition. Analysis of variance (ANOVA) was used as the statistical analytical tool. The results indicated that the solvent type significantly influenced the phytochemical profile of the Moringa extracts. 70% Ethanol extract showed the highest concentration of secondary metabolites such as cardiac glycosides, alkaloids, saponins, tannins, phenols, flavonoids anthraquinones, sterols & Terpenoids, followed by *Water* extract which was also rich in water-soluble nutrients, such as cardiac glycosides, saponins, tannins, & flavonoids. *Ethylacetate*, which is can both extract polar and non-polar compounds, was next in yield, and extracted secondary metabolites such as cardiac glycosides, alkaloids, saponins, tannins, phenols, flavonoids, anthraquinones, sterols, & Terpenoids, while *N-Hexane* extract, being more effective at extracting non-polar compounds, contained the least yield, and extracted secondary metabolites such as anthraquinones, sterols, & Terpenoids. The study found that the ethanol extract of *Moringa oleifera* leaf exhibited the highest% Yield, suggesting that ethanol could be an ideal solvent for extracting a broad range of bioactive compounds. In conclusion, this study highlights the importance of solvent selection in the extraction of phytochemicals from *Moringa oleifera* leaf and provides valuable insights into optimizing extraction protocols for pharmaceutical applications. Further research is needed to explore the synergy between solvent combinations and their effects on the therapeutic efficacy of Moringa extracts.

Keywords: *Moringa oleifera*, ethanol, water, ethylacetate, N-hexane

Introduction
Moringa oleifera, commonly known as the "drumstick tree" or "miracle tree," has gained significant attention due to its impressive nutritional and medicinal properties. The plant is rich in vitamins, minerals, antioxidants, and other bioactive compounds, making it valuable in traditional medicine and a potential source of various bioactive molecules with therapeutic benefits [1]. The leaves of *Moringa oleifera*, in particular, are renowned for their high concentrations of nutrients such as vitamins A, C, and E, minerals like calcium, potassium, and iron, as well as protein and essential amino acids. In addition to these essential nutrients, Moringa leaves contain a wide variety of phytochemicals, including flavonoids, phenolic acids, alkaloids, and saponins, which contribute to its medicinal properties [2]. Phytochemicals, which are naturally occurring compounds in plants, are known to have a broad range of biological activities such as anti-inflammatory, antioxidant, antimicrobial, and anticancer effects.

The extraction of these compounds from plant materials is a critical step in evaluating their biological potential, and the choice of solvent used in the extraction process plays a significant role in determining the types and quantities of phytochemicals that can be obtained [3]. Different solvents-such as water, ethanol, methanol, chloroform, and acetone-have varying polarities, which influence their ability to dissolve specific phytochemicals from plant tissues [4].

Polar solvents like water and methanol are typically effective in extracting water-soluble compounds such as flavonoids, phenolic acids, and alkaloids, whereas non-polar solvents like chloroform and hexane are better suited for extracting lipophilic compounds such as essential oils and fatty acids [5]. Thus, the selection of an appropriate extraction solvent is crucial in maximizing the yield of bioactive compounds from Moringa leaves and ensuring the efficacy of the extraction for both nutritional and medicinal purposes [6].

The effect of different extraction solvents on the phytochemical profile of *Moringa oleifera* leaves has not been comprehensively explored, and there is limited information regarding how solvent choice might impact the antioxidant, anti-inflammatory, or antimicrobial properties of the extracted compounds [7]. This study aims to investigate the effects of various extraction solvents on the phytochemical constituents of *Moringa oleifera* leaves, with the goal of identifying the most efficient solvent or solvent mixture for obtaining a wide range of bioactive compounds from the leaves.

The findings of this research could help optimize extraction methods for the development of Moringa-based pharmaceuticals, nutraceuticals, and functional foods. Furthermore, understanding the relationship between solvent choice and phytochemical yield could aid in selecting the most appropriate extraction method for specific therapeutic applications, thus enhancing the potential uses of *Moringa oleifera* in both traditional and modern medicine [8].

Materials and Methods: Plant Collection: The leaf of *Moringa oleifera* L. was collected from Rayfield, in Jos South Local Government Area of Plateau State, Nigeria in April 2024. The identity of the plant was authenticated in the Federal College of Forestry, Jos and voucher number, FCF 221 was given. The herbarium specimen was then deposited in the Department of Pharmacognosy & Traditional Medicine, University of Jos.

Plant Preparation

The sampled leaves were garbled and dried under shade for 3 weeks. The dried leaves were then ground into small particles using a commercial blender (John-Morris Scientific, Chats-wood, NSW, Australia) and then sieved using a steel mesh sieve (1.4 mm EFL 2000; Ende-cotts Ltd., London, England) and stored in an air-tight amber bottle. The powdered kept at −20 °C until when needed for further analysis.

Chemicals and reagents

All the solvents used in the study were of Analytical grade.

Extraction

The drug powder (1kg) was sequentially extracted with hexane (3L), ethyl acetate (3L) methanol (3L) and water (3L) by maceration/electric shaking for 72 hours in each case. The extracts were concentrated under reduced pressure using Rota-vapor to have Hexane extract (3.03% yield), Ethyl acetate extract (19.01% yield) and 70% ethanol extract (20.40% yield) and water extract (10.00%).

Phytochemical Screening

The different extracts were subjected to phytochemical screening for the presence of chemical constituents such as alkaloids, saponins, flavonoids, tannins, cardiac glycosides, anthraquinones, phenols, and steroids using standard procedures [1, 3, 14].

Statistical analysis

The results of the comparative analysis were expressed as mean ± Standard Deviation (SD) using one-way Analysis of variance (ANOVA). Differences were considered statistically significant at $p<0.05$.

Results

Table 1: Results of the% yield of different extracts *Moringa oleifera* leaf

Extract	% Yield
Water	10.00
70% Ethanol	20.40
Ethyl- acetate	19.01
N-Hexane	03.03

Table 2: Results of the phytochemical screening of the different extracts *Moringa oleifera* leaf

Test/ Extract	C. Glycosides	alkaloids	Saponins	Tannins	phenols	Flavonoids	Anthra-quinones	sterols	terpenoids
Water	+	-	+	+	-	+	-	-	-
70% Ethanol	+	+	+	+	+	+	+	+	+
Ethyl- acetate	+	+	+	+	+	+	+	+	+
N-Hexane	-	-	-	-	-	-	+	+	+

Discussion

Four different solvents were selected based on their polarity. They included polar solvents: water and 70% ethanol; an intermediate solvent: ethylacetate; and a non-polar solvent: N-hexane. Ethanol extract was found to contain the highest percentage yield of secondary metabolites. Ethanol, a polar solvent yet was able to extracts both polar compounds (c. glycosides, alkaloids, saponins, tannins, phenols, and flavonoids), and non-polar compounds (anthraquinones, sterols, and terpenoids). This anomalous behavior of ethanol is simply due to the reason that, it is considered a universal solvent [1, 3, 7, 11, 13, 15]. The molecular structure of ethanol allows it to dissolve both polar and nonpolar compounds [1, 3, 7, 11, 13, 15]. Ethanol is a versatile solvent that mixes with

water and many other organic solvents, including benzene, acetone, ethylene, glycol, chloroform, toluene, glycerol, nitromethane, carbon tetrachloride, pyridine, and diethyl ether [1, 2, 3, 7, 9, 11, 13, 15]. Ethanol is a polar compound with a hydroxyl group that makes it reactive [1, 2, 3, 7, 9, 11, 13, 15]. It has a number of unique properties, including the fact that it shrinks in volume when mixed with water; and expands in volume when mixed with gasoline; etc. [1, 2, 3, 7, 9, 11, 13, 14].

Ethylacetate extract was the next closest in terms of percentage yield and the number of secondary metabolites. Ethylacetate can be both polar and non-polar because it contains both polar and non-polar groups: it has polar carbonyl (c=o) and oxygen groups and a non-polar ethyl-group [1, 2, 3, 5, 6, 7, 9, 11, 13, 15]. It is a moderately polar organic solvent that is used for dissolving a wide range of compounds [1, 2, 3, 5, 6, 7, 9, 11, 13, 15]. It is often used in analytical chemistry to separate and purify analytes from complex matrices [1, 2, 3, 5, 6, 7, 9, 11, 13, 15].

Water extracted only polar compounds such as c. glycosides, alkaloids, saponins, tannins, phenols, and flavonoids. Water is a polar solvent because, it has a slight negative charge near its oxygen atom and a slight positive charge near its hydrogen atoms [1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15]. The oxygen atom in water is more electronegative than the hydrogen atoms, so it pulls the shared electrons closer to it. This gives water an uneven distribution of electron density, making it a polar molecule [1, 2, 3, 5, 6, 7, 9, 11, 13, 15].

Furthermore, because of its polarity, water can be attracted to the positive or negative charges of other molecules. This allows water to dissolve many different types of molecules, making it an excellent solvent [1, 2, 3, 5, 6, 7, 9, 11, 13, 15]. Water molecule are also held together by hydrogen bonds, which form between the hydrogen of one water molecule and the oxygen of another. That means that, the unequal sharing of electrons makes water a polar molecule. This makes the oxygen end of the molecule slightly negative. Since the electrons are not near the hydrogen end as much, that end is slightly positive. When a covalently bonded molecule has more electrons in one area than another, it is called a polar molecule [1, 2, 3, 5, 6, 7, 9, 11, 13, 15].

N-hexane extract gave the least percentages yield with anthraquinones, sterols and terpenoids as secondary metabolites. That is because it is a non-polar solvent and are only attracted to non-polar compounds [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. N-hexane is made up of atoms with similar electronegativities, which results in bonds that lack partial charges [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. This means that, it is hydrophobic and can only extract polar compounds. It contains a non-polar carbon-hydrogen bonds, making it highly non-polar [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. While there is a slight difference in electronegativity between its hydrogen and carbon atoms, it is not significant enough to be polar. Its symmetrical structure would cancel the polarity even if polar bonds were in the molecule [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15].

Conclusion

Polarity of solvents is important for optimal extraction efficiency. This study has filled this knowledge gap by comparing the effects of various extraction solvents on the phytochemical constituents of *Moringa oleifera* leaf. The findings of this research could significantly improve the extraction process and contribute to the sustainable development of Moringa-based products with enhanced therapeutic potential.

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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