

Identifying the phytochemical content in *Illicium verum* (Star Anise) extracts prepared with different polarity solvents based on a simple maceration method

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Abstract

This study focuses on identifying the phytochemical composition of Star anise (*Illicium verum*) extract with different polarity solvents, including n-hexane, distilled water, and methanol by using a maceration method. The percentage yield of compounds extracted was obtained highest by methanol (29.6615 %), followed by distilled water (19.1831%) and n-hexane (11.5475%). Standard phytochemical tests were conducted to elucidate the phytochemical profile further. The results revealed significant variations in the phytochemical content among the extracts obtained with different solvents. The foam, alkaline reagent, and ferric chloride tests confirmed the presence of saponins, flavonoids and tannins, and phenolic compounds, respectively. Specifically, methanol extract exhibited a higher concentration of phenolics, tannins, and flavonoid compounds compared to distilled water extract, while n-hexane extract showed superior content of saponins. These findings underscore the importance of solvent selection in optimizing the extraction of specific phytochemicals from *Illicium verum*. Overall, this study provides comprehensive insights into the phytochemical constituents of *Illicium verum*, essential for understanding its medicinal properties and potential applications in various industries.

1. Introduction

Star anise, formally known as *Illicium verum*, has a long history in traditional Chinese medicine, serving as both a spice and an herbal remedy. The plant is native to southern China and Vietnam, but it is also grown in other regions with adequate conditions. The spice is derived from the *Illicium verum* tree, which can grow 6 to 8 meters tall. It features glossy, evergreen leaves and small yellow blooms. All the tree's parts have a nice, fragrant scent. *Illicium verum* has an identified licorice-like flavor due to its high concentration of anethole, the same compound responsible for the flavor of actual anise seeds (Wang *et al.*, 2011). However, *Illicium verum* has a more intense, sweeter flavor profile compared to anise seeds. The name "star anise" refers to the star-shaped fruit, which is collected and dried before usage. Its extracts, which mostly include trans-anethole (85-90%), as well as compounds like estragole and anisaldehyde, have been extensively studied (Pu *et al.*, 2014). Notably, *Illicium verum* and its extracts have a particular aromatic profile typical of aromatic plants. *Illicium verum* and its extracts are well-known for their safety and therapeutic benefits, which include antiparasitic, antibacterial,

antioxidant, antifungal, and antipyretic properties. These bioactive compounds serve a significant role in lowering environmental stress, as evidenced by in vivo and in vitro studies (Patra *et al.*, 2020).

Even while it may not be as effective as other extraction methods for extracting specific classes of compounds or reaching high yields, maceration extraction is a simple, gentle, and less disruptive process, allowing for efficient extraction of phytochemicals than boiling and distillation. Fortunately, it remains a popular approach for extracting phytochemical substances from natural sources like plants, herbs, and seeds due to maintaining simplicity and cost-effectiveness (Palai *et al.*, 2022). The maceration method involves soaking the plant material in a suitable solvent for an extended period to allow the solvent to dissolve the desired compounds. During this time, the solvent slowly permeates the plant material, dissolving the soluble constituents and extracting their flavors, aromas, and therapeutic properties. The extraction process is generally quite long and requires days or even weeks to complete, depending on the desired strength and characteristics of the final extract (Abubakar *et al.*, 2020).

However, the polarity of the solvent utilized during extraction can have a substantial impact on the phytochemical profile and bioactivity of the extracts produced. Phytochemicals, which include phenolics, alkaloids, terpenoids, and flavonoids, have varying polarities. Polar liquids have molecules with an unequal charge distribution, indicating the presence of both positive and negative poles (Asman *et al.*, 2023). These solvents are beneficial for extracting polar phytochemicals such as phenolic compounds, flavonoids, and alkaloids, which contain functional groups such as hydroxyl (-OH), carbonyl (C=O), and amino (-NH₂) (Mustapha *et al.*, 2023). Examples of polar solvents include water, ethanol, methanol, and acetone. Water is typically used to extract polar molecules because of its high polarity and capacity to establish hydrogen bonds with phytochemical functional groups. Nonpolar solvents have molecules with a more evenly distributed charge and no polar functional groups. These solvents are useful for extracting nonpolar phytochemicals such as terpenoids, essential oils, and lipids (Lefebvre *et al.*, 2021). Nonpolar solvents include ethyl acetate, chloroform, n-hexane, and diethyl ether. Nonpolar solvents are very beneficial for extracting substances like essential oils, which are made up of nonpolar hydrocarbons (Yara-Varón *et al.*, 2017). The polarity of the solvent influences the selectivity of the extraction process. Polar solvents preferentially extract polar molecules, whereas nonpolar solvents extract nonpolar chemicals. Therefore, the choice of solvent can be tailored to the specific phytochemicals of interest. Understanding the polarity of the solvents and the target phytochemicals is critical for designing effective extraction methods.

Phytochemical screening is a systematic technique that uses several chemical assays to detect and analyze the presence of numerous bioactive substances, or phytochemicals, in plant extracts (Velavan *et al.*, 2015). The screening procedure can aid in the identification of several phytochemical groups, including alkaloids, flavonoids, terpenoids, saponins, tannins, phenols, glycosides, and others. Each category of phytochemicals may have unique properties and health implications. The screening method may comprise a variety of assays, such as color reactions, precipitation reactions, or specific chemical reactions, to observe characteristic changes or reactions that indicate the presence of a given phytochemical group. It is important to note that phytochemical screening only offers qualitative information about the presence or absence of certain chemicals, not their quantities (Nagano *et al.*, 2021).

This study aims to identify the phytochemical composition in *Illicium verum* extracts prepared with different polarity solvents including n-hexane, distilled water, and methanol based on a simple maceration method. The phytochemical compounds present in the *Illicium verum* extracts were elucidated by phytochemical tests. Furthermore, based on the various polarity solvents used, the percentage yield of extraction and the outcome of phytochemical extraction present in *Illicium verum* were compared. This present work lies in its contribution to the understanding of the phytochemical composition of *Illicium verum* extracts based on different polarity solvents using the maceration method. The adoption of a simple maceration method is significant as it provides a low-cost and easily accessible method for extracting phytochemicals.

2. Material and Methods

2.1 Materials

Star anise (*Illicium verum*) was bought from a shop in Pagoh Jaya, Muar. Methanol (99.9 wt.%, LabServ), n-hexane (95%), and distilled water were used as solvents for extraction. Ferric chloride (Sigma-Aldrich) solution and sodium hydroxide (98%, Emory) solution were used for the phytochemical screening test.

2.2 Preparation sample using maceration extraction

Firstly, the *Illicium verum* was pounded into powder. Then, 5 grams of powdered *Illicium verum* were macerated with three different 100 ml of solvents individually: distilled water, methanol, and n-hexane for four days. Then, the extracted *Illicium verum* was filtered using gravity filtration, and the filter solid residue was dehydrated in the oven for 24 hours at 70 °C. The amount of filter solid residue was weighed and recorded. Meanwhile, the

Illicium verum extracts were tested through phytochemical screening. This procedure was adopted by Shaikh et al. (2020). Fig. 1 presents the dried extraction solid residual during the experiment.



Fig. 1 The dried extraction solid-residual (n-hexane, methanol and distilled water from the left side)

2.3 Identification of saponin using foam test

10 drops of distilled water were mixed with a small quantity of sample extracts in the test tube. Then, the mixture was shaken for 10 minutes, and the foam-producing sample was observed.

2.4 Identification of flavonoid using alkaline reagent test

In the test tube, a small quantity of sample extract was mixed with three drops of 1.0 M sodium hydroxide solution. The yellow-colored producing sample was observed.

2.5 Identification of phenolic and tannin compounds using ferric chloride test

Firstly, 5 grams of ferric chloride powder was weighed and dissolved in 100 ml of distilled water. Following that, 3 drops of the prepared ferric chloride solution were mixed with a small amount of sample extracts in the test tube. Color alterations from brownish green to blue-black were noted.

2.6 Preparation of percentage yield extraction of *Illicium verum*

Prior to maceration, the initial weight of the *Illicium verum* was measured. The remaining *Illicium verum* residue from the maceration process was air-dried for 5 days at room temperature. The dried residue was then weighed to determine the percentage yield. The percentage yield of each extraction from three distinct solvents was determined using Eq. 1.

$$\text{Percentage yield (\%)} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100 \quad (1)$$

3. Result and Discussion

3.1 Percentage yield of *Illicium verum* extracts

Table 1 shows the weight loss of the *Illicium verum* from different solvents. The methanol solvent result showed the highest percentage of yield with 29.6615% compared to the distilled water (19.1831%) and n-hexane (11.5475%). This is because methanol is the polar solvent with good solubility that allows it to dissolve in polar and non-polar compounds. A polar solvent with both positive and negative polarities enables it to dissolve a broad spectrum of compounds and thus has the broadest extracting range (Oktavianawati *et al.*, 2023). It can be effective for extracting certain substances and compounds especially organic and natural product compounds (Fotsing Yannick Stéphane *et al.*, 2022). Distilled water is also a polar solvent that can be an excellent solvent for certain compounds such as polar compounds and is not suitable for extraction of insoluble and low solubility water compounds. The n-hexane solvent showed the lowest percentage of yield compared to the two solvents. It can be summarized that the phytochemical content and extraction yield are significantly influenced by the polarity solvent used. The extraction with highly polar solvents obtained a higher extract yield compared to less polar solvents. It is possible to increase the effectiveness of qualitative and quantitative phytochemical extraction from various natural plants by employing a mixture of polar and nonpolar solvents specifically (Ng *et al.*, 2023; Azizuddin *et al.*, 2023).

Table 1 Percent yield of *Illicium verum*

Solvent	Initial weight of <i>Illicium verum</i> (g)	Final weight of <i>Illicium verum</i> (g)	Percentage yield (%)
Distilled water	5.0070	4.0465	19.1831%
Methanol	5.0166	3.5286	29.6615%
n-hexane	5.0383	4.4565	11.5475%

3.2 Phytochemical screening of *Illicium verum*

Screening of phytochemicals is a method that determines the presence of specific classes of phytochemicals using a suitable screening reagent. The presence classes of phytochemicals determined for this work are tannins, phenolics, flavonoids, and saponins. The reaction of the phytochemical present in *Illicium verum* extract solution with the specific reagent can be observed through the physical change of the solution like the form of the foam, color changes of the solution, and the forming of precipitate. Fig. 2(a) shows the forming of the foam in the form of gel or cloudy solution. The forming of foam occurred in the extraction solution of methanol and n-hexane solvent and did not occur in the extraction solution of distilled water solvent. The presence of phenolics and tannins compounds can be determined through the ferric chloride test in Fig. 2(b). It is proved that the phenolics and tannins compound presented in the extraction solution of methanol and distilled water solvent by changing the color of the solution to a brownish black solution and no changes occurred in the extraction solution of n-hexane solvent because of the polarity difference. In Fig. 2(c), the alkaline reagent test shows the extraction solution changes to the yellowish color of the solution when there is the presence of a flavonoid compound. The result proved the presence of flavonoid compounds in the extraction solution of distilled water and methanol solvent and not the presence of flavonoids in the extraction solution of n-hexane. Table 2 summarizes the presence of phytochemical content in *Illicium verum*. The findings were referred to the study by Khadeeja and Faris (2023).

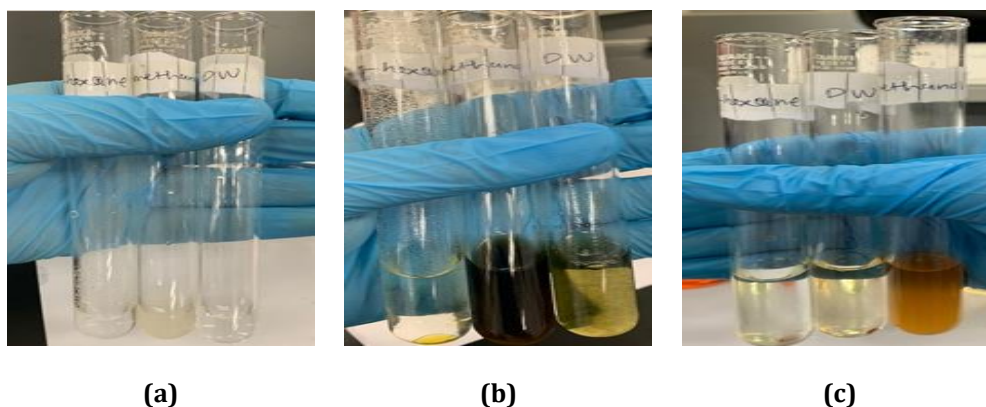


Fig. 2 The results of (a) the foam test to determine the presence of saponins, (b) the ferric chloride test to determine the presence of phenolics and tannins, (c) the alkaline reagent test to determine the presence of flavonoids

Table 2 Result of phytochemical screening in *illicium verum* for three different solvents.

Phytochemicals	Methanol	Distilled water	n-Hexane
Saponins	+	-	++
Phenolics	++	+	-
Tannins	++	+	-
Flavonoids	++	+	-

Footnotes: (-) indicates absence, (+) indicates presence at good concentration, (++) indicates presence at high concentration.

4. Conclusion

The various phytochemicals were successfully identified in *Illicium verum*. The maceration extraction technique was applied by using methanol, distilled water, and n-hexane within the order percentage of yield obtained: methanol > distilled water > n-hexane due to polarity influence, indicating that highest polarity of methanol is the best solvent for *Illicium verum* extracts. Besides, the difference of these solvents indicated different results in the phytochemical content of *Illicium verum*. The resulting *Illicium verum* extract by distilled water resulted in the presence of flavonoids, tannins, and phenolics. The resulting *Illicium verum* extract by methanol resulted in the flavonoids, tannins, phenolic, and saponins present, and n-hexane obtained the presence of flavonoids and saponins. It was summarized that the significance of solvent selection in improving the extraction of particular phytochemicals from *Illicium verum* is crucial. It gives thorough insights into the phytochemical elements of *Illicium verum*, which are critical for understanding its therapeutic characteristics and possible applications in a variety of industries. The identification of the specific phytochemicals compounds found in *Illicium verum* extract will be further investigated specifically by using methanol as the main chosen solvent will be highlighted.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: **study conception and writing:** Alya Athirah Mohd Idris; **guidance and made corrections:** Alya Athirah Mohd Idris and Saliza Asman; **Reviewer:** Saliza Asman. All authors reviewed the results and approved the final version of the manuscript.

References

- [1] Abubakar, A., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy And Bioallied Sciences*, 12(1), 1. https://doi.org/10.4103/jpbs.jpbs_175_19
- [2] Asman, S., & Mohd Zin, S. R.. (2023). Extraction Of Phytochemicals In *Morinda citrifolia* L. Leaves By Using Different Polarity Solvents. *Enhanced Knowledge in Sciences and Technology*, 3(2), 286–291. Retrieved from <https://publisher.uthm.edu.my/periodicals/index.php/ekst/article/view/13784>
- [3] Azizuddin, H.F., Nordin, N.F., Saiman, S. K, Asman, S & Mohd Idris, A.A. (2023). Extraction of phytochemicals from *Murraya Koenigii* (L.) Spreng leaves using maceration method. *Scientific Research Journal*, 20(2), 174-160. <https://ir.uitm.edu.my/id/eprint/85035>
- [4] Fotsing Yannick Stéphane, F., Kezet Jean Jules, B., El-Saber Batiha, G., Ali, I., & Ndjakou Bruno, L. (2022). Extraction of bioactive compounds from medicinal plants and herbs. *Natural Medicinal Plants*. <https://doi.org/10.5772/intechopen.98602>
- [5] Khadeeja, T.A., & Faris, T.A. (2023). Phytochemical comparative studies, antioxidant and antimicrobial of artemisia and star anise. *Pharmacognosy Journal*, 13. <https://www.phcogj.com/article/1979>
- [6] Lefebvre, T., Destandau, E., & Lesellier, E. (2021). Selective extraction of bioactive compounds from plants using recent extraction techniques: A Review. *Journal of Chromatography A*, 1635, 461770. <https://doi.org/10.1016/j.chroma.2020.461770>
- [7] Mustapha, T., Ithnin, N. R., Othman, H., Abu Hasan, Z.-'Iffah, & Misni, N. (2023). Bio-fabrication of silver nanoparticles using citrus aurantifolia fruit peel extract (CAFPE) and the role of plant extract in the synthesis. *Plants*, 12(8), 1648. <https://doi.org/10.3390/plants12081648>
- [8] Ng, H.Y., Gan, S.M & Saliza, A. (2023). Extraction of essential oil *Citrus hystrix* by using ultrasound-assisted extraction. *Malaysian Journal of Analytical Sciences*, 27(6), 1236-1248.
- [9] Oktavianawati, I., Santoso, M., & Fatmawati, S. (2023). Metabolite profiling of Borneo's *gonystylus bancanus* through comprehensive extraction from various polarity of solvents. *Scientific Reports*, 13(1). <https://doi.org/10.1038/s41598-023-41494-7>

- [10] Palai, S., Shekhawat, N. (2022). Novel Extraction Techniques for Phytochemicals: A Comprehensive Review International Journal of Pharmaceutical Research (09752366), 14
<https://doi.org/10.31838/ijpr/2022.14.03.004>
- [11] Patra, J. K., Das, G., Bose, S., Banerjee, S., Vishnuprasad, C. N., del Pilar Rodriguez-Torres, M., & Shin, H. (2020). Star Anise (*illicium verum*): Chemical compounds, antiviral properties, and clinical relevance. *Phytotherapy Research*, 34(6), 1248–1267.
<https://doi.org/10.1002/ptr.6614>
- [12] Pu, Xu. Thermal reaction of anisaldehyde in the presence of l-cysteine, a model reaction of Chinese stew meat flavor generation. Retrieved from
<https://doi.org/doi:10.7282/T3MP51KH>
- [13] Sayuri Nagano, M., & Batalini, C. (2021). Phytochemical screening, antioxidant activity and potential toxicity of azadirachta indica A. Juss (neem) leaves. *Revista Colombiana de Ciencias Químico-Farmacéuticas*, 50(1).
<https://doi.org/10.15446/rcciquifa.v50n1.95447>
- [14] Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603–608.
<https://doi.org/10.22271/chemi.2020.v8.i2i.8834>
- [15] Velavan S. (2015) Phytochemical Techniques - A Review. *World Journal of Science and Research*. 1(2): 80-91
- [16] Wang, G.-W., Hu, W.-T., Huang, B.-K., & Qin, L.-P. (2011). *Illicium verum*: A review on its botany, traditional use, chemistry, and pharmacology. *Journal of Ethnopharmacology*, 136(1), 10–20.
<https://doi.org/10.1016/j.jep.2011.04.051>
- [17] Yara-Varón, E., Li, Y., Balcells, M., Canela-Garayoa, R., Fabiano-Tixier, A.-S., & Chemat, F. (2017). Vegetable oils as alternative solvents for green oleo-extraction, purification and formulation of food and natural products. *Molecules*, 22(9), 1474.
<https://doi.org/10.3390/molecules22091474>