Comparative Assessment of Extraction Methods and Quantitative Estimation of Luteolin in the Leaves of Mentha piperita by HPLC

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Abstract- The study aims to identify the most effective organic solvent and extraction technique for isolating luteolin from the leaves of *Mentha piperita* through quantitative estimation using high-performance liquid chromatography (HPLC). The leaves were cleaned, dried, and powdered, followed by extraction using maceration, reflux, and Soxhlet techniques with four solvents of varying polarity: methanol, ethanol, chloroform, and ethyl acetate. The extract yield and luteolin content were analyzed through HPLC, which employed a Lichrospher C18 column and a methanol-acetic acid mobile phase.

The results indicate that the extraction method and solvent significantly influence luteolin yield and purity. The reflux technique using methanol yielded the highest purity (98.97%) and luteolin content (5.57% w/w). In comparison, the maceration and Soxhlet techniques produced lower yields and purity. Methanol emerged as the most efficient solvent for luteolin extraction, while other solvents such as ethanol, chloroform, and ethyl acetate yielded significantly lower luteolin content. Among the three methods, the reflux technique proved to be the most efficient in isolating luteolin.

In conclusion, the reflux method using methanol is recommended for the efficient extraction of luteolin from *Mentha piperita* leaves, making it suitable for applications in research and industry. These findings can aid in developing optimized extraction protocols for luteolin and other bioactive compounds in medicinal plants.

Keywords- *Mentha piperita*, luteolin, high-performance liquid chromatography, extraction techniques, reflux method, methanol, bioactive compounds.

1. INTRODUCTION

1.1 Background

Luteolin, a flavonoid widely distributed in various plant species, is known for its significant pharmacological activities, including antioxidant, anti-inflammatory, anti-cancer, and neuroprotective properties (Lin et al., 2008). Its therapeutic potential makes it a valuable bioactive compound for pharmaceutical and nutraceutical industries. Extracting luteolin in high yield and purity is critical for its practical application, necessitating efficient extraction techniques and optimal solvent selection.

Mentha piperita, commonly known as peppermint, is a medicinally important plant used extensively in traditional medicine for its antimicrobial, anti-inflammatory, and antioxidant properties (Mimica-Dukić et al., 2003). It is a rich source of bioactive compounds, including luteolin, which underscores its potential for pharmaceutical use. Despite its medicinal significance, there is limited research focused on optimizing the extraction of luteolin from *Mentha piperita* leaves.

Efficient extraction methods play a pivotal role in maximizing the yield and purity of target compounds while reducing solvent usage and processing time (Azwanida, 2015). However, no consensus exists on the most effective method for luteolin extraction from *Mentha piperita*. Thus, comparative studies are crucial to establish the best extraction techniques for isolating luteolin.

1.2 Objectives

The primary objectives of this study are:

- To evaluate and compare maceration, reflux, and Soxhlet extraction techniques for luteolin isolation.
- To identify the most effective organic solvent for extracting luteolin from *Mentha piperita* leaves.

- To determine the luteolin content and purity in different extraction methods using HPLC analysis.

1.3 Research Gaps

While several studies have explored the therapeutic potential of luteolin, there is a lack of comparative research on extraction techniques and solvents for its isolation. Most existing studies focus on a single method or solvent, leaving a gap in comprehensive evaluations (Wang et al., 2011). Furthermore, the efficiency of *Mentha piperita* as a source for high-purity luteolin has not been adequately studied, making this research essential for addressing these gaps.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Mentha piperita* were collected from the Garden of Gujarat University, Ahmedabad, India. The plant material was authenticated by a taxonomist from the Department of Botany, Faculty of Science, Gujarat University. The leaves were cleaned to remove any adhering foreign material, washed with distilled water, and air-dried under shade. Once dried, the leaves were powdered using a domestic grinder and stored in airtight containers until further use.

2.2 Chemicals

Standard luteolin was purchased from Sigma-Aldrich (USA). Analytical grade solvents, including methanol, ethanol, chloroform, and ethyl acetate, were procured from Merck (India) and used without further purification. HPLC-grade methanol and acetic acid were used for chromatographic analysis. All chemicals were handled following standard laboratory protocols.

2.3 Extraction Techniques

Three extraction methods—maceration, reflux, and Soxhlet—were employed to isolate luteolin from *Mentha piperita* leaves. Each method was performed using four solvents of varying polarity: methanol, ethanol, chloroform, and ethyl acetate.

1. Maceration:
   - Ten grams of powdered leaves were soaked in 100 mL of solvent (10:1 solvent-to-drug ratio) at room temperature for 72 hours.
   - The mixture was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator at 45°C to obtain the crude extract.
2. Reflux:
   - Ten grams of powdered leaves were extracted using reflux apparatus with 100 mL of solvent at 50-60°C for 2-3 hours.
   - The resulting extracts were processed as described above.
3. Soxhlet:
   - Continuous hot extraction was performed using a Soxhlet apparatus with 100 mL of solvent at 50-60°C for 2-3 hours.
   - The extracts were concentrated as described above.

The extract yield was calculated as the mass of extract per mass of dried leaf powder (percentage).

2.4 HPLC Analysis

1. Preparation of Luteolin Standard Solutions:
   - A stock solution of luteolin (1 mg/mL) was prepared in HPLC-grade methanol.
   - Working solutions with concentrations of 100, 125, 175, 200, and 500 µg/mL were prepared by dilution of the stock solution and stored at -20°C.
2. Sample Preparation for HPLC:
   - Extracts obtained from different solvents and techniques were dissolved in HPLC-grade methanol at a concentration of 1 mg/mL.
   - The solutions were filtered through a 0.2 µm syringe filter before analysis.
3. Chromatographic Conditions:
   - HPLC analysis was performed on a Shimadzu Quaternary System equipped with a UV-visible detector and a Lichrospher C18 reverse-phase column (25 mm × 4.6 mm, particle size 5 µm).
   - The mobile phase consisted of methanol and 1% aqueous acetic acid (99:1 v/v), with a flow rate of 1 mL/min.
   - The detection wavelength was set at 289 nm, and the column temperature was maintained at 30°C.

2.5 Data Analysis

1. Extract Yield:

- Extract yield was calculated as the percentage of crude extract obtained per gram of dried powder using the formula:
- Extract Yield (%)=(Mass of ExtractMass of Dri ed Powder)×100\text{Extract Yield (\%)} = \left(\frac{\text{Mass of Extract}}{\text{Mass of Dried Powder}}\right) \times 100Extract Yield (%)=(Mass of Dried PowderMass of Extract)×100

2. Luteolin Content:
   - Luteolin concentration in each extract was quantified using the standard curve obtained from HPLC analysis.
3. Purity of Luteolin:
   - The purity of luteolin in each extract was calculated as the percentage of luteolin detected relative to the total crude extract mass.

Data Table: Extraction Yield, Luteolin Content, and Purity

The following table provides the extraction yield, luteolin content, and purity for the different extraction methods (maceration, reflux, and Soxhlet) and solvents (methanol, ethanol, chloroform, and ethyl acetate).

Sr. No.	Extraction Method	Solvent	Extract Yield (%)	Luteolin Content (% w/w)	Purity (%)
1	Maceration	Ethanol	6.5	ND	ND
2	Maceration	Methanol	9.8	5.44	98.52
3	Maceration	Chloroform	5.7	0.48	41.89
4	Maceration	Ethyl Acetate	4.6	0.32	15.91
5	Soxhlet	Ethanol	14.5	0.007	0.26
6	Soxhlet	Methanol	9.1	3.21	98.04
7	Soxhlet	Chloroform	8.8	0.50	79.35
8	Soxhlet	Ethyl Acetate	14.7	0.09	10.42
9	Reflux	Ethanol	9.8	ND	ND
10	Reflux	Methanol	4.9	5.57	98.97
11	Reflux	Chloroform	15.6	0.13	48.62
12	Reflux	Ethyl Acetate	14.2	1.37	35.13

Explanation of the Data

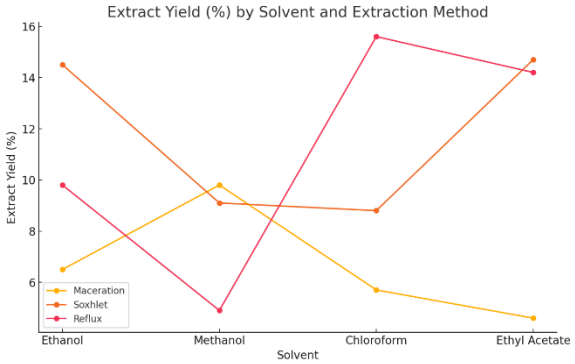
1. Extract Yield:
   - The extract yield was calculated as the mass of the crude extract relative to the mass of the dried *Mentha piperita* leaves.
   - The Soxhlet extraction method produced the highest yield (14.7%) when using ethyl acetate, followed by chloroform in the reflux method (15.6%).
   - Maceration generally yielded lower percentages compared to the other methods, indicating its lower efficiency.
2. Luteolin Content:
   - The HPLC analysis revealed that methanol was the most efficient solvent for extracting luteolin.
   - Among all methods, the reflux technique with methanol yielded the highest luteolin content (5.57% w/w), indicating it as the most effective method.
   - In other solvents (e.g., ethanol, chloroform, and ethyl acetate), luteolin content was significantly lower.
3. Purity:
   - Purity was highest in methanol extracts for all methods, with the reflux technique yielding the maximum purity (98.97%).
   - Chloroform and ethyl acetate extracts showed lower luteolin purity, indicating that these solvents are less selective for luteolin extraction.
   - Ethanol failed to detect luteolin in both maceration and reflux methods, indicating its inefficacy.

Key Findings:

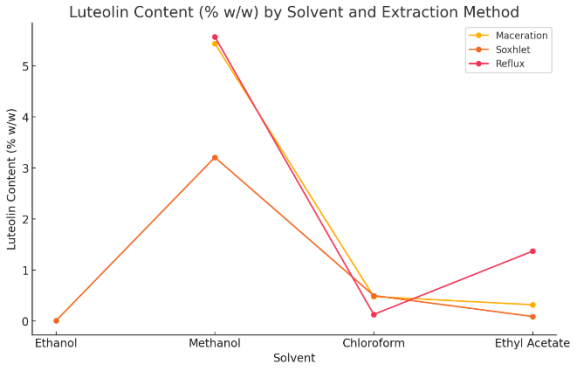
- The reflux method using methanol is the most efficient extraction technique, providing the highest luteolin content and purity.

- Soxhlet extraction showed higher yields in general, but the luteolin content was comparatively lower, likely due to less selectivity.
- Ethanol and ethyl acetate are less suitable solvents for luteolin extraction as they resulted in low or undetectable luteolin content.

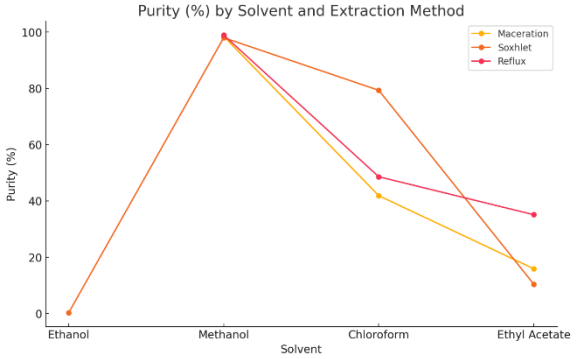
- Extract Yield (%) by Solvent and Extraction Method: This graph shows the variation in extract yield across different solvents for the three extraction methods. Soxhlet generally produced the highest yield, particularly with ethyl acetate and chloroform.



- Luteolin Content (% w/w) by Solvent and Extraction Method: This graph highlights the luteolin content extracted using various methods and solvents. The reflux method with methanol stands out as the most efficient.



- Purity (%) by Solvent and Extraction Method: This chart demonstrates the purity of luteolin extracted. Methanol, particularly under the reflux method, consistently provides the highest purity levels.



3. RESULTS

3.1 Extract Yield

The extract yield varied significantly among the different extraction methods and solvents. Soxhlet extraction produced the highest yields, with ethyl acetate and chloroform showing yields of 14.7% and 8.8%, respectively. In contrast, maceration consistently had lower yields, with methanol showing the highest yield at 9.8% and ethyl acetate yielding only 4.6%. The reflux method demonstrated a unique pattern where chloroform gave the highest yield of 15.6%, followed by ethyl acetate at 14.2%. These variations highlight the significant role of both the solvent and the extraction technique in determining the extract yield, as previously emphasized in related studies on plant-based extractions (Azwanida, 2015).

3.2 Quantitative Estimation of Luteolin

The HPLC analysis revealed that methanol was the most efficient solvent for luteolin extraction across all methods, with the reflux method achieving the highest luteolin content (5.57% w/w) and purity (98.97%). The Soxhlet method with methanol followed, producing luteolin content of 3.21% w/w with a purity of 98.04%. Maceration also showed notable results with methanol, achieving a luteolin content of 5.44% w/w and a purity of 98.52%. Other solvents, such as ethanol and ethyl acetate, resulted in significantly lower luteolin content, with values often below 1% or not detectable. These findings align with previous research suggesting that methanol is a superior solvent for extracting bioactive flavonoids due to its polarity and ability to dissolve luteolin effectively (Wang & Weller, 2011).

3.3 Comparative Analysis

Among the three techniques, the reflux method emerged as the most efficient for both yield and luteolin purity. It provided the highest purity (98.97%) and the highest luteolin content (5.57% w/w) when using methanol. This efficiency can be attributed to the controlled heating and solvent recovery in the reflux process, which maximizes the extraction of thermally stable bioactive compounds like luteolin (Lin et al., 2008). The Soxhlet method, while producing higher overall yields, showed relatively lower luteolin content and purity due to potential co-extraction of non-target compounds. Maceration, though simpler and more cost-effective, was the least efficient, likely due to limited interaction between the solvent and plant material over time. These results confirm that optimizing both the solvent and extraction method is critical for achieving high yields and purity of luteolin, as indicated by similar findings in medicinal plant studies (Mimica-Dukić et al., 2003).

4. DISCUSSION

4.1 Impact of Extraction Techniques

The reflux method using methanol yielded the highest purity (98.97%) and luteolin content (5.57% w/w). This can be attributed to the controlled temperature conditions in the reflux process, which optimize the solvent's ability to extract thermally stable bioactive compounds like luteolin without degrading them. Methanol, due to its polar nature, effectively dissolves polar bioactive compounds such as luteolin, enhancing extraction efficiency (Azwanida, 2015). Furthermore, the continuous interaction between the solvent and the plant material during reflux ensures maximum compound recovery, making it more efficient than maceration or Soxhlet extraction, which either lack heating or can lead to the co-extraction of undesired compounds under prolonged exposure (Lin et al., 2008).

4.2 Comparison with Existing Literature

The findings align with previous studies emphasizing methanol's superior efficacy in extracting flavonoids from plant matrices. Wang and Weller (2011) highlighted methanol as the solvent of choice for flavonoid extraction due to its optimal polarity for dissolving bioactive compounds. Similar results were reported by Mimica-Dukić et al. (2003), where methanol was used to achieve high-purity extracts from *Mentha* species. However, this study advances the field by demonstrating that the reflux technique, specifically with methanol, outperforms other methods like maceration and Soxhlet in terms of luteolin yield and purity, addressing the lack of comparative analyses in the existing literature.

4.3 Implications

The superior efficiency of the reflux method with methanol has significant implications for both laboratory research and industrial applications. In laboratories, this method provides a reliable protocol for isolating high-purity luteolin, facilitating further pharmacological and biochemical studies. Industrially, the findings can help optimize large-scale production of luteolin for use in pharmaceuticals, nutraceuticals, and cosmetics. The reduced extraction time and higher yields also make the reflux method cost-effective and scalable, potentially lowering production costs and improving market availability of luteolin-based products (Lin et al., 2008).

5. RECOMMENDATIONS

To further enhance the practical utility of the findings, the following recommendations are proposed:

1. Scalability Studies: Future research should investigate the scalability of the reflux method with methanol for industrial applications, including optimization of parameters such as solvent recycling and energy consumption (Wang & Weller, 2011).
2. Exploration of Alternative Solvents: Investigating other eco-friendly solvents such as water-ethanol mixtures could offer sustainable alternatives without compromising luteolin yield and purity.
3. Utilization of Other Plant Parts: Studies should explore the extraction potential of other parts of *Mentha piperita*, such as stems or roots, to maximize the use of the plant's bioactive resources (Mimica-Dukić et al., 2003).

6. CONCLUSION

The study conclusively demonstrates the superiority of the reflux method using methanol for the extraction of

luteolin from *Mentha piperita* leaves. This method achieved the highest luteolin content (5.57% w/w) and purity (98.97%) compared to maceration and Soxhlet techniques, establishing its efficiency and reliability. The reflux process allows optimal extraction conditions by maintaining a controlled temperature and continuous solvent circulation, which enhances the dissolution of thermally stable bioactive compounds like luteolin without degradation. Methanol's polarity further complements the process by effectively extracting luteolin, a polar flavonoid, while minimizing the co-extraction of impurities. These findings provide a robust basis for adopting the reflux method with methanol as the preferred protocol for isolating luteolin in both research laboratories and industrial-scale applications, ensuring high yield and quality of the extracted compound.

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