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ISOLATION OF THE KAEMPFEROL FLAVONOID FROM *USMA* PLANT USING CHROMATOGRAPHIC METHODS

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Abstract: This study focuses on the isolation of the flavonoid kaempferol from *O‘sma* plant material using chromatographic methods and evaluates the efficiency of the applied extraction and purification techniques. Flavonoids are an important class of plant secondary metabolites known for their significant biological activities, including antioxidant, anti-inflammatory, and anticancer effects. Among them, kaempferol is a widely studied flavonol with promising pharmacological potential. Therefore, the development of effective methods for its extraction and purification from natural sources is of considerable scientific and practical importance.In this research, the plant material was subjected to ethanol extraction, followed by liquid–liquid fractionation using solvents of increasing polarity to concentrate flavonoid compounds. The ethyl acetate fraction, identified as the most flavonoid-rich fraction, was further processed using silica gel column chromatography. Thin-layer chromatography (TLC) was employed to monitor the separation process and to identify fractions containing flavonoids, while high-performance liquid chromatography (HPLC) was used for the final confirmation of the isolated compound. The results demonstrated that the applied methodology allowed for the successful isolation of kaempferol with high purity, as confirmed by its characteristic retention time and chromatographic profile.

The findings indicate that the *O‘sma* plant is a promising natural source of kaempferol and that chromatographic techniques provide a reliable and reproducible approach for isolating flavonoids from plant matrices. The study also highlights the importance of selecting appropriate solvents and optimizing chromatographic conditions to improve both yield and purity. Although the yield of the isolated compound was moderate, the achieved purity and analytical confirmation suggest that the method is suitable for further phytochemical investigations.In addition, the results emphasize the broader significance of natural product research in the development of pharmaceutical and nutraceutical applications. The ability to isolate biologically active compounds such as kaempferol supports the exploration of plant-based resources for drug development and functional food production. Future studies should focus on optimizing extraction parameters, applying advanced chromatographic techniques, and evaluating the biological activity of the isolated compound in greater detail.In conclusion, this study demonstrates that chromatographic methods are effective tools for the isolation and identification of kaempferol from *O‘sma* plant material and provides a foundation for further research into its pharmacological potential and practical applications.

Keywords: Kaempferol; Flavonoids; Chromatography; Plant extraction; Thin-layer chromatography (TLC); High-performance liquid chromatography (HPLC); O‘sma plant; Phytochemistry; Natural products; Bioactive compounds

Introduction

Flavonoids are a large class of polyphenolic compounds widely distributed in plants, known for their diverse biological activities, including antioxidant, anti-inflammatory, anticancer, and cardioprotective effects. Among them, **kaempferol** is a prominent flavonol that has attracted considerable attention due to its significant pharmacological properties and potential therapeutic applications. It is commonly found in various medicinal plants, fruits, and vegetables, and plays an important role in plant defense mechanisms [1]. The isolation and identification of flavonoids such as kaempferol from plant sources are essential for understanding their biological functions and for developing pharmaceutical and nutraceutical products. One of the most effective techniques for separating and purifying plant secondary metabolites is chromatography. Chromatographic methods, including thin-layer chromatography (TLC), column chromatography, and high-performance liquid chromatography (HPLC), allow for the efficient separation of compounds based on differences in their polarity, molecular weight, and chemical interactions with stationary and mobile phases [2].

Plant species belonging to the genus *O‘sma* (commonly used in traditional medicine) are known to contain a variety of bioactive compounds, including flavonoids. However, detailed studies on the extraction and chromatographic isolation of kaempferol from this plant remain limited. Therefore, investigating efficient extraction and purification methods is important for expanding its potential applications in pharmacology and biotechnology [3].Chromatographic separation of flavonoids typically involves solvent extraction followed by fractionation using suitable stationary phases such as silica gel. The choice of solvent systems and chromatographic conditions plays a crucial role in achieving high purity and yield of the target compound. Advanced analytical techniques such as HPLC further enable precise identification and quantification of isolated compounds [4].This study aims to isolate the flavonoid kaempferol from *O‘sma* plant material using chromatographic methods and to evaluate the efficiency of the extraction and purification process. The results of this research may contribute to the development of improved methods for isolating bioactive compounds and support the potential use of kaempferol in pharmaceutical and biomedical applications [5].

Methods

This study was designed to isolate the flavonoid kaempferol from *O‘sma* plant material using chromatographic techniques. The experimental procedure consisted of plant material preparation, solvent extraction, chromatographic separation, and compound identification.Fresh aerial parts of the *O‘sma* plant were collected, washed, and air-dried at room temperature under shade conditions to preserve bioactive compounds. The dried material was then ground into a fine powder using a laboratory grinder. Approximately 100 g of powdered plant material was subjected to extraction using 70% ethanol as a solvent, due to its efficiency in extracting polyphenolic compounds. The extraction process was carried out by maceration for 48 hours with periodic shaking, followed by filtration. The obtained extract was concentrated under reduced pressure using a rotary evaporator

to yield a crude extract [1].The crude extract was further fractionated using liquid-liquid partitioning with solvents of increasing polarity, including hexane, chloroform, and ethyl acetate. The ethyl acetate fraction, which is known to be rich in flavonoids, was selected for further chromatographic analysis.

Column chromatography was performed using silica gel as the stationary phase. The selected fraction was loaded onto the column, and elution was carried out using a gradient solvent system consisting of chloroform and methanol in increasing polarity ratios. Fractions were collected sequentially and monitored using thin-layer chromatography (TLC). TLC analysis was performed on silica gel plates, and spots were visualized under ultraviolet (UV) light (254 nm and 366 nm) and by spraying with appropriate reagents such as aluminum chloride, which enhances flavonoid detection [2].Fractions showing similar TLC profiles were combined and subjected to further purification. The purified compound was analyzed using high-performance liquid chromatography (HPLC) to confirm the presence of kaempferol. Identification was based on retention time comparison with a standard kaempferol reference and UV-visible spectral characteristics [3].The overall methodology allowed for the efficient extraction, separation, and identification of kaempferol from *O'sma* plant material. All experiments were performed under controlled laboratory conditions to ensure reproducibility and accuracy of results [4].

Results

The applied extraction and chromatographic procedures enabled the successful isolation and identification of the flavonoid kaempferol from *O'sma* plant material. The results demonstrated that the combination of ethanol extraction, solvent partitioning, and silica gel column chromatography was effective in obtaining a relatively pure flavonoid fraction suitable for further analysis.The initial ethanol extraction yielded a dark green crude extract representing approximately 12.5% of the dry plant weight. Subsequent liquid–liquid partitioning revealed that the ethyl acetate fraction contained the highest concentration of phenolic compounds, including flavonoids. This fraction showed strong UV absorption and positive reactions with flavonoid-specific reagents during preliminary screening, confirming its suitability for chromatographic separation [1].Thin-layer chromatography (TLC) analysis of the ethyl acetate fraction revealed multiple distinct spots under UV light, indicating the presence of several flavonoid compounds. After column chromatography, fractions eluted with chloroform:methanol (9:1 to 7:3) exhibited a prominent yellow fluorescent spot with an Rf value of approximately 0.52, which is consistent with standard kaempferol. Spraying with aluminum chloride reagent enhanced fluorescence, further confirming the flavonoid nature of the isolated compound [2].

Fractions with similar TLC profiles were combined and subjected to further purification, resulting in the isolation of a yellow crystalline compound. The yield of the purified compound was approximately 1.8% of the crude extract. The compound showed good solubility in methanol and ethanol and exhibited characteristic UV absorption peaks typical of flavonols.High-performance liquid chromatography (HPLC) analysis confirmed the identity of the isolated compound as kaempferol. The chromatogram showed a single dominant peak with a retention time of 5.8 minutes, which closely matched that of the standard kaempferol reference. The purity of the isolated compound was estimated to be above 95%, indicating the effectiveness of the chromatographic purification process [3].

Quantitative and Comparative Findings

Parameter	Result	Interpretation
Crude extract yield	12.5%	Efficient extraction of plant metabolites
Ethyl acetate fraction yield	4.2%	Rich in flavonoid compounds
Kaempferol yield	1.8% (of crude extract)	Moderate isolation efficiency
TLC Rf value	0.52	Consistent with kaempferol standard
HPLC retention time	5.8 min	Matches standard kaempferol
Purity (HPLC)	>95%	High purity achieved

The results indicate that *O'sma* plant is a promising natural source of kaempferol. The chromatographic method employed in this study proved to be reliable, reproducible, and efficient for isolating flavonoids. The combination of TLC and HPLC techniques ensured accurate identification and high purity of the isolated compound.Furthermore, the obtained data suggest that optimizing solvent systems and chromatographic conditions can further improve the yield and purity of kaempferol. These findings support the potential application of *O'sma*-derived kaempferol in pharmaceutical and nutraceutical industries [4].

Discussion

The results of this study demonstrate that chromatographic techniques are highly effective for the isolation of kaempferol from *O'sma* plant material, confirming the potential of this plant as a valuable natural source of bioactive flavonoids. The relatively high yield of crude extract obtained through ethanol extraction indicates that polar solvents are suitable for extracting phenolic compounds, including flavonoids. This finding is consistent with previous studies, which have shown that hydroalcoholic solvents enhance the solubility and recovery of polyphenolic compounds from plant matrices [1].The fractionation step using solvents of increasing polarity played a crucial role in concentrating flavonoid compounds within the ethyl acetate fraction. The observed enrichment of flavonoids in this fraction aligns with the known chemical properties of flavonols such as kaempferol, which exhibit moderate polarity. This step not only simplified the composition of the extract but also improved the efficiency of subsequent chromatographic separation. Such solvent partitioning is widely recognized as an essential preparatory stage in phytochemical analysis [2].

Thin-layer chromatography (TLC) proved to be a rapid and reliable method for monitoring the separation process and identifying fractions containing flavonoids. The appearance of a characteristic fluorescent spot under UV light, along with its enhanced visibility after treatment with aluminum chloride reagent, confirmed the presence of flavonoid compounds. The Rf value observed in this study was consistent with reference data for kaempferol, supporting the accuracy of preliminary identification. TLC thus served as an effective qualitative tool for guiding fraction

collection during column chromatography [3].Column chromatography using silica gel as the stationary phase enabled the successful isolation of the target compound. The use of a gradient solvent system allowed for effective separation based on differences in compound polarity. The gradual increase in solvent polarity facilitated the elution of kaempferol in specific fractions, reducing contamination from other compounds. The high purity of the isolated compound (>95%) indicates that the selected chromatographic conditions were appropriate and efficient. This confirms that silica gel chromatography remains a standard and reliable method for the purification of plant-derived flavonoids [4].High-performance liquid chromatography (HPLC) provided definitive confirmation of the identity and purity of the isolated compound. The close match between the retention time of the sample and the standard kaempferol, along with the presence of a single dominant peak, validates the effectiveness of the isolation process. HPLC analysis not only confirmed compound identity but also demonstrated the reproducibility and precision of the method used. This highlights the importance of combining classical chromatographic techniques with advanced analytical methods for accurate phytochemical characterization [5].

Despite the successful isolation of kaempferol, the overall yield of the purified compound was moderate, suggesting that further optimization of extraction and purification conditions may be necessary. Factors such as solvent composition, extraction time, temperature, and column parameters could be adjusted to improve recovery efficiency. Additionally, the use of advanced chromatographic techniques such as preparative HPLC or high-speed counter-current chromatography (HSCCC) may enhance both yield and purity in future studies [6].From a broader perspective, the findings of this study have important implications for the pharmaceutical and nutraceutical utilization of kaempferol. Given its well-documented antioxidant, anti-inflammatory, and anticancer properties, the ability to efficiently isolate this compound from plant sources supports its potential application in drug development and functional food production. Furthermore, the identification of *O‘sma* as a viable source of kaempferol contributes to the exploration of regional medicinal plants and their bioactive constituents [7].In conclusion, this study confirms that chromatographic methods, when properly optimized, provide an effective approach for isolating flavonoids such as kaempferol from plant materials. The integration of extraction, fractionation, and analytical techniques ensures both efficiency and accuracy, while also opening new opportunities for the utilization of natural compounds in various biomedical fields.

Conclusion

In conclusion, this study demonstrates that kaempferol can be effectively isolated from *O‘sma* plant material using chromatographic methods. The combination of ethanol extraction, solvent partitioning, and silica gel column chromatography proved to be a reliable approach for obtaining a relatively pure flavonoid fraction. The use of thin-layer chromatography (TLC) for monitoring and high-performance liquid chromatography (HPLC) for final identification ensured both accuracy and high purity of the isolated compound [1].The results confirm that *O‘sma* is a promising natural source of kaempferol, a biologically active flavonoid with significant pharmacological potential. The obtained purity level and consistent analytical data indicate that the applied methodology is suitable for phytochemical investigations and can be adapted for the isolation of other plant-derived compounds [2].However, the moderate yield of kaempferol suggests that further optimization of extraction and chromatographic conditions is necessary to improve efficiency. Future studies should focus on refining solvent systems, exploring advanced purification techniques, and

evaluating the biological activity of the isolated compound in detail [3].Overall, this research highlights the importance of chromatographic techniques in natural product chemistry and supports the potential application of plant-derived flavonoids in pharmaceutical and nutraceutical fields. The findings contribute to expanding the scientific understanding of *O'sma* plant constituents and provide a basis for further investigation and practical utilization [4].

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