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Comparative Study of Aqueous Extract of Tulsi and Mulhethi Using HPLC

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Abstract:

In this study, we aimed to extract and analyze the bioactive compounds present in leaves of Tulsi (*Ocimum sanctum*) and Mulethi (*Glycyrrhiza glabra*) these plants, focusing on the identification and quantification of quercetin, a flavonoid with diverse pharmacological activities using high-performance liquid chromatography (HPLC). Quercetin, a flavonoid present in both plant extracts, utilizing high-performance liquid chromatography (HPLC), was located and measured. Moreover, a calibration curve was created using standard quercetin solutions. This study highlighted the differences in chemical constituents between the two plants.

Keywords: Tulsi, Mulethi, HPLC, Quercetin, Flavonoids.

1. Introduction:

Herbal medicine refers to using leaves of plants, flowers, seeds and roots of plants for medicinal purpose which is also known as Botanical Medicine and Phytomedicine (Nikhil et al., 2021). The well-known medicinal plants Tulsi (*Ocimum sanctum*) and Mulethi (*Glycyrrhiza glabra*) have been used for centuries in traditional medical systems. They have a wide variety of bioactive substances that add to their medicinal qualities (Parida JR., 2019). Understanding the therapeutic potential of the plants and determining the precise elements that contribute to their biological activities depend on the extraction and evaluation of these compounds from the aqueous extracts of the plants (Bhattacharya S, 2018).

Tulsi, commonly referred to as holy basil, has a significant role in Ayurveda, the conventional Indian medical system. It is regarded as a sacred plant and has long been valued for its many therapeutic benefits. Antioxidant, immunomodulatory antibacterial, and anti-inflammatory properties of tulsi are well-known. It includes a variety of bioactive substances that support its pharmacological actions, including phenols, flavonoids, and essential oils (Dang LH., 2019). A recent study by Lopresti et al. 2022 conclude that supplementation with extract of tulsi lowers stress and improves sleep quality.

Another plant that has been widely used in traditional medicine by several civilizations is mulethi, also referred to as licorice. It has a long history of therapeutic uses and is renowned for its hepatoprotective, expectorant, anti-inflammatory, and antiulcer qualities. Glycyrrhizin, liquiritin, and glabridin are the main bioactive components of Mulethi, and they all contribute to its therapeutic properties (Gupta M., 2018).

Clinical and experimental research suggests that it possesses anti-inflammatory, antiviral, immunomodulatory, hepatoprotective, antimicrobial, antioxidant, anticancer, and cardioprotective activities (Chandran et al., 2022).

One of the most important steps in using medicinal plants for therapeutic reasons is the extraction of their bioactive components (Mondal S., 2016). A popular technique for getting plant extracts that are abundant in water-soluble chemicals is aqueous extraction. This technique entails soaking the plant material in water to release the soluble components into the solution. Traditional medicine prefers aqueous extracts because of their safety and simplicity of use (Jena J., 2019).

Analytical methods with the ability to distinguish between, recognize, and quantify distinct components are necessary for evaluating the bioactive chemicals in plant extracts. In phytochemical analysis, chromatography methods like high-performance liquid chromatography (HPLC) have become essential instruments (Jia Y. 2018). Based on their physicochemical characteristics, complex mixtures can be divided into their separate components using HPLC. It is the perfect technology for the examination of plant extracts since it has great resolution, sensitivity, and reproducibility (Joshi RK., 2019).

The extraction and assessment of the aqueous extracts of tulsi and mulethi are the main topics of this study. To ensure their purity and to get rid of any impurities, these plants' leaves are collected, cleaned, and dried. The extraction procedure is then made easier by first powdering the dried leaves (Karthikeyan A., 2019).

Suitable extraction methods, such as the Soxhlet equipment, are used to do the aqueous extraction. With this technique, the bioactive substances from the plant material are extracted effectively. The resulting concentrated aqueous extracts can be further examined for their chemical make-up and bioactive elements (Kundu S., 2017).

High-performance liquid chromatography (HPLC) is used to analyze the aqueous extracts of Mulethi and Tulsi. This method permits an identification and separation of specific chemicals found in the extracts. Specific compounds can be located and measured by comparing the resultant chromatograms with recognized standards and calibration curves (Lin SS., 2015).

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Insights into the chemical make-up and potential therapeutic uses of Tulsi and Mulethi are gained by the extraction and analysis of bioactive components from their aqueous extracts. Our knowledge of the therapeutic benefits of these plants is improved when we are aware of the presence and concentration of specific chemicals, such as phenols, flavonoids, and other bioactive elements. This information also helps us create evidence-based therapeutic interventions (Mishra A., 2020).

2. Material and Methodology

2.1 Plant collection

The process of collecting Mulethi (*Glycyrrhiza glabra*) and Tulsi (*Ocimum sanctum*) leaves for extraction took place in an herbal garden located in Gohana, Sonipat district of Haryana, India. To ensure the purity of the leaves and eliminate any potential contaminants from their natural environment, the collected leaves were carefully washed using tap water, effectively removing dust and other unwanted materials. After that washed in distilled water.

After the washing process, the leaves were protected from direct sunlight and placed in a shaded area to dry naturally at room temperature. This drying period typically lasts for approximately 4-5 days, allowing the leaves to achieve the desired dryness level. Once the leaves were dried, they were finely ground into a powder using a grinding apparatus. The powder weight was also measured and recorded for accurate dosing purposes during the extraction process. To preserve the quality and potency of the powdered Mulethi and Tulsi leaves, a clean and tightly sealed container was utilized for storage. By following these meticulous steps, the Mulethi and Tulsi leaves were prepared and ready for extraction. This ensured that the resulting aqueous extract would be of the highest possible quality. The extract was then filtered and stored in an airtight container to keep it fresh and free from contamination.

2.2 Plant extract preparation

To prepare the aqueous solvent extraction from Mulethi and Tulsi leaves, the Soxhlet apparatus was employed. By employing the Soxhlet apparatus and utilizing an aqueous solvent, the extraction process ensured the efficient extraction of bioactive compounds from Mulethi and Tulsi leaves. This method provided a concentrated and labelled aqueous extract that could be further utilized for various experiments and investigations.

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2.3 HPLC Analysis

The HPLC analysis of the aqueous Tulsi and Mulhethi leaf extracts was carried out and performed by the technical staff of Dr A.P.J Abdul Kalam - CIL Lab from Guru Jambheshwar University of Science and Technology, Hisar. The sample volume used for injection in to the column was 20µl and the flow rate was 1.0ml/min. The mobile phase used in this system comprised acetonitrile and water.

2.4 Identification of marker compound (Quercetin)

In this analytical method, a reverse-phase C-18 column was employed, which achieved a steady state in the mobile phase. The mobile phase consisted of a 50:50 (v/v) solution of distilled water and acetonitrile. Before use, the mobile phase was filtered using Whatman filter paper and degassed to remove air bubbles. The mobile phase flow rate was maintained at 1 mL per minute, and the effluents were monitored at 256 nm. To analyse the sample extracts, a fixed loop with a volume of 10 µl was used. The injection was performed for 10 minutes during the entire run. By subjecting the plant extract samples to chromatography using this HPLC method, the concentration of quercetin in both plant extracts was determined based on a regression equation. This research used HPLC to investigate and quantify the flavonoid component, specifically quercetin, in selected medicinal plants.

2.5 Preparation of the calibration curve of the quercetin

To construct the calibration curve, standard quercetin solutions were prepared and injected into the column three times. The standard drug peak area was measured for each injection, and the mean peak area was calculated. The concentration of the standard drug solutions ranged from 5 µg/ml to 25 µg/ml, and distilled water was used as the solvent system. Using the data obtained from the standard solutions, a calibration curve was plotted by graphing the peak area against the different concentrations of the drug. The regression equations were derived based on the curve, allowing drug quantification in subsequent analyses. The calibration curve obtained from the standard solution was then used to compare the peaks observed in the sample extracts of Tulsi and Mulhethi.

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2.6 Identification of quercetin in two different extracts of the plants

A qualitative analysis of quercetin in selected phytoextracts was conducted using the HPLC method.

2.7 Preparation of the calibration curve of carbohydrate (Maltose)

To construct the calibration curve, standard maltose solutions were prepared and injected into the column three times. Further same procedure will be conducted as mentioned above in case of Quercetin calibration curve preparation.

3. Results:

Calibration curve of Quercetin:

By comparing the peak areas of the sample extracts with the calibration curve, the concentration of quercetin in the extracts could be determined (**Table 1.1, Figure 1.1)**

Table 1.1: Preparation of calibration curve

S.No.	Concentration (µg/ml)	Absorbance
1.	5	401.151
2.	10	871.271
3.	15	1369.167
4.	20	1872.986
5.	25	2239.219

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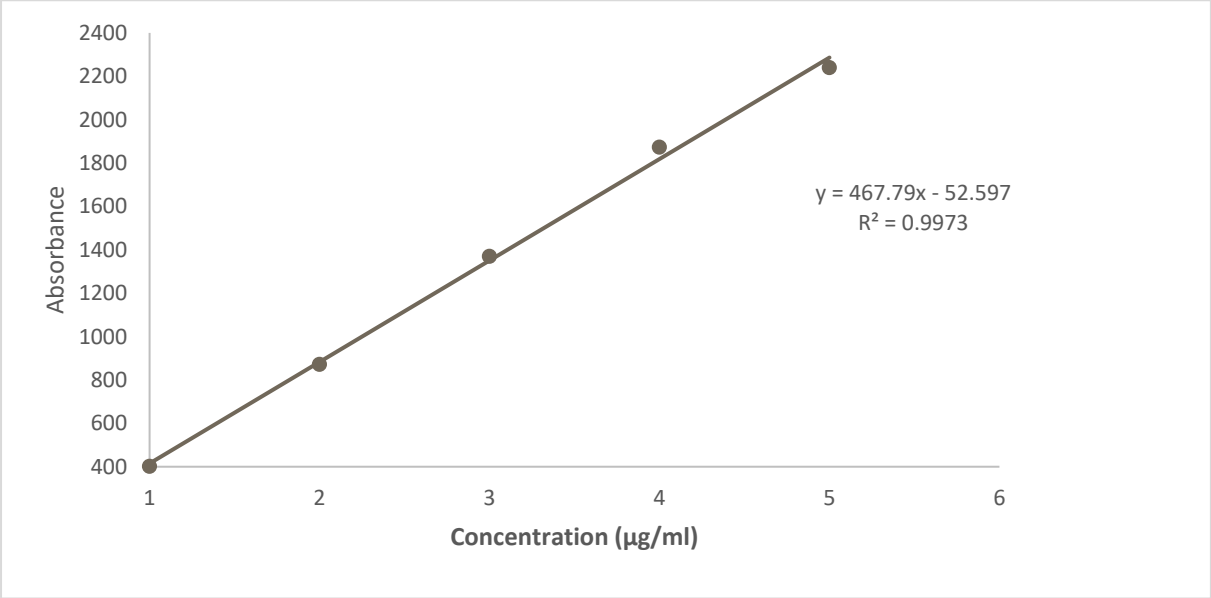

Figure 1.1: Calibration Curve of the Quercetin

The calibration curve for flavonoids displayed a linear relationship within the concentration range of 5, 10, 15, 20, and 25 µg/ml, with a high coefficient of determination (R^2 = 0.99). The linear regression equation for the curve was expressed as y = 467.79x - 52.597, where y represents the peak area ratio of flavonoids and x corresponds to the concentration of flavonoids in µg/ml.

Identification of quercetin in two different extracts of the plants

A qualitative analysis of quercetin in selected phytoextracts was conducted using the HPLC method. The HPLC chromatogram of the aqueous extracts from Tulsi and Mulhethi leaves showed prominent peaks indicating quercetin as a secondary metabolite. The resulting HPLC chromatograms of the standard quercetin and the plant extract samples were recorded and are presented in the tables below. This study further confirmed the presence of flavonoids (quercetin) as secondary metabolites in both medicinal plants.

Table 1.2 Characteristics of the analytical method derived from the standard calibration curve

Compound	Linearity range (µg/ml)	Correlation coefficient	Slope	Intercept
Quercetin	5-25	0.997	467.79	52.597

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The chromatographic analysis of aqueous leaf extracts from plants was performed using a mobile phase consisting of acetonitrile and aqueous 50:50 (V/V) solutions. The flow rate used was 1 ml per minute, and the absorbance was measured at 256 nm. This method resulted in effective quercetin separation, with a retention time of 2.50 minutes. In the HPLC chromatogram of Tulsi leaf extracts, a higher peak area was observed at a retention time of 2.54 minutes. Similarly, the HPLC fingerprints of Mulhethi leaf extracts revealed significant peaks at a retention time of 2.56 minutes at a wavelength of 256 nm. Both medicinal plants contained quercetin. The chromatographic peaks of quercetin in the plant extracts were compared to the retention time (RT) values obtained from the chromatogram of the standard quercetin, using similar experimental conditions. The standard quercetin exhibited a strong peak at 2.59 minutes. HPLC analysis of the plants' aqueous leaf extracts revealed different peak areas at the same retention times. This is depicted in the figures. However, the results indicated that the peaks of various plant extracts did not significantly differ from the standard quercetin, further confirming its presence. Comparative chromatographic analysis of both plants demonstrated quercetin presence in both species. This study highlighted the differences in chemical constituents between the two plants, with a slight variation observed in the flavonoid (quercetin) component (Figure 1.2, Figure 1.3, and Figure 1.4).

HPLC for Carbohydrate (Maltose)

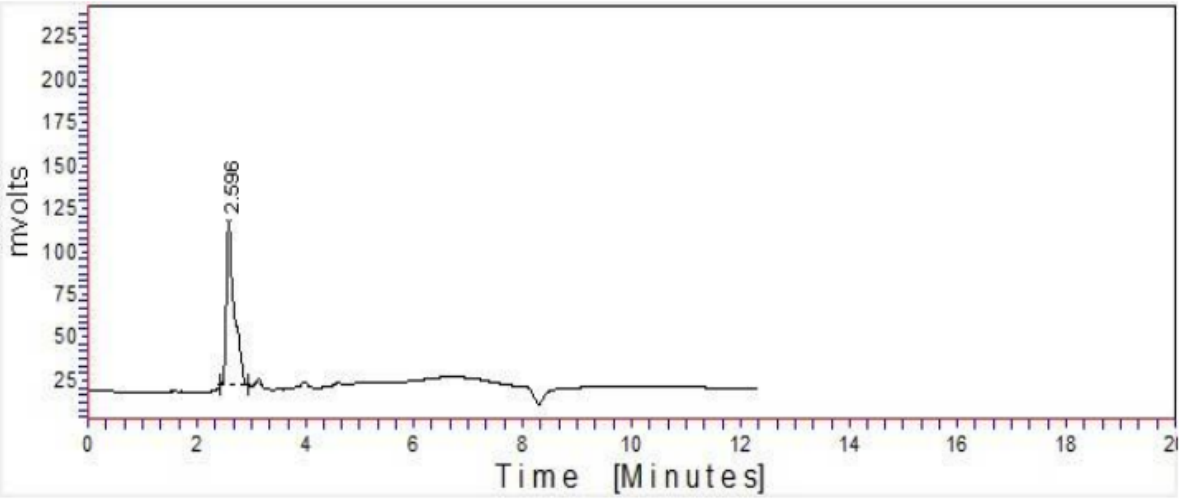

Figure 1.2: Chromatogram of standard Quercetin

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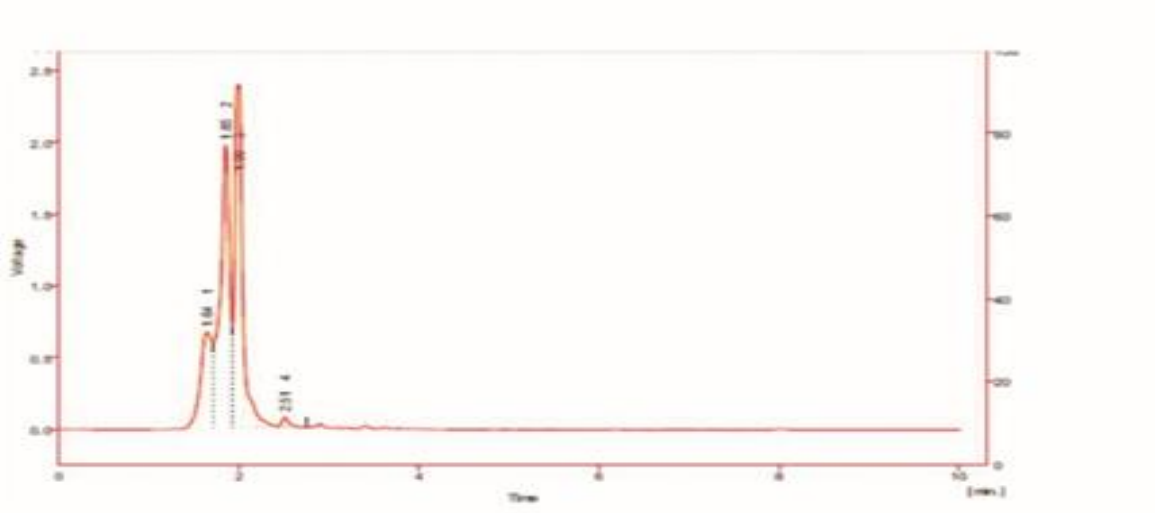

Figure 1.3: Chromatogram ofTulsi leaf extract

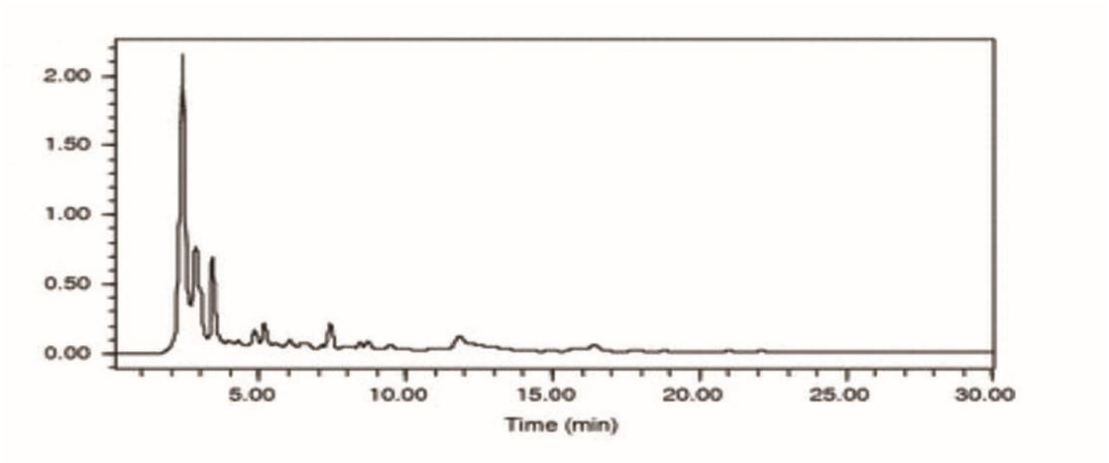

Figure 1.4: Chromatogram of Mulhethi leaf extract

3.2 Calibration curve of carbohydrate (Maltose)

Using the data obtained from the standard solutions, a calibration curve was plotted by graphing the peak area against the different concentrations of the drug. The regression equations were derived based on the curve, allowing drug quantification in subsequent analyses. The calibration curve obtained from the standard solution was then used to compare the peaks observed in the sample extracts of Tulsi and Mulhethi. By comparing the peak areas of the sample extracts with the calibration curve, the concentration of quercetin in the extracts could be determined (**Table 1.3, Figure 1.5)**

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Table 1.3: Preparation of calibration curve

S.No.	Concentration (µg/ml)	Absorbance
6.	5	274.231
7.	10	411.251
8.	15	576.361
9.	20	725.452
10.	25	887.937

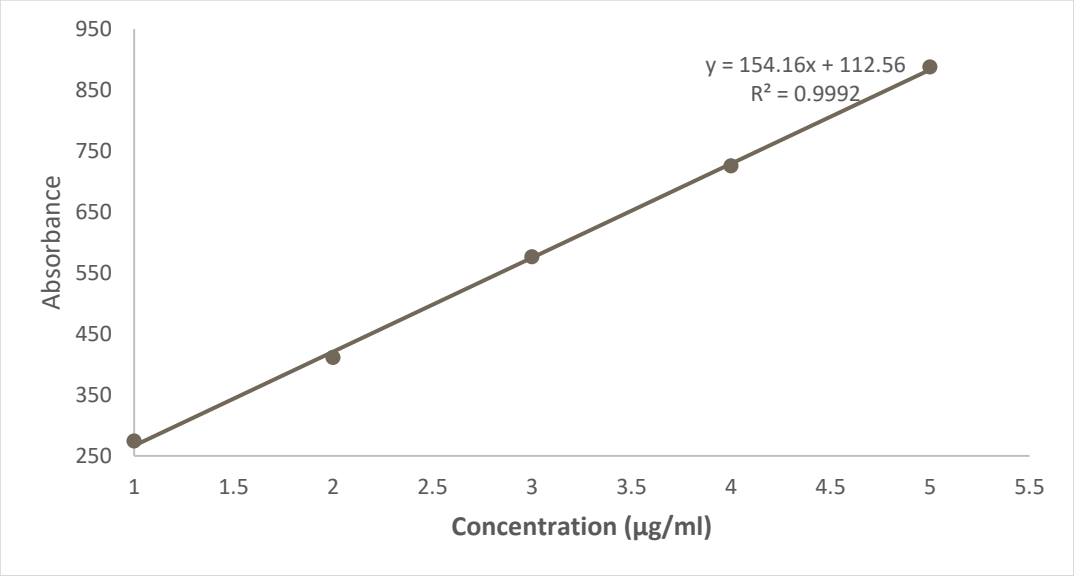

Figure 1.5: Calibration Curve of the carbohydrate (Maltose)

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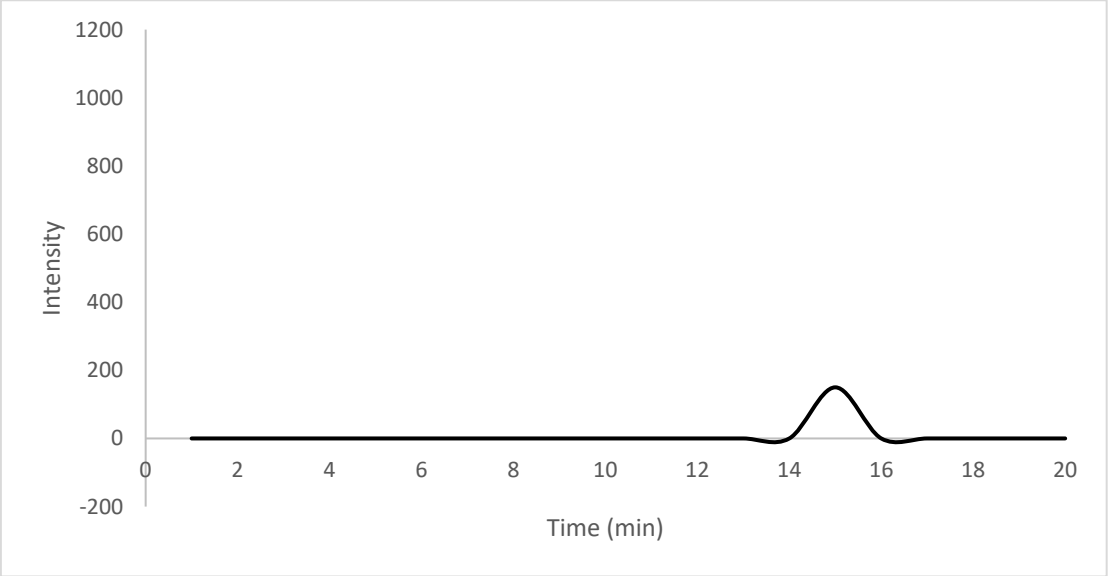

Figure 1.6: Chromatogram of standard carbohydrate (Maltose)

The calibration curve for maltose displayed a linear relationship within the concentration range of 5, 10, 15, 20, and 25 µg/ml, with a high coefficient of determination (R^2 = 0.99). The linear regression equation for the curve was expressed as y = 154.16x + 112.56, where y represents the peak area ratio of flavonoids and x corresponds to the concentration of flavonoids in µg/ml.

Identification of Maltose in two different extracts of the plants

A qualitative analysis of quercetin in selected phytoextracts was conducted using the HPLC method. The HPLC chromatogram of the aqueous extracts from Tulsi and Mulhethi leaves did not show any peaks indicating carbohydrate (maltose). The resulting HPLC chromatograms of the standard maltose and the plant extract samples were recorded and are presented in the table 1.3. This study further confirmed the presence of flavonoids (quercetin) as secondary metabolites in both medicinal plants.

Table 1.3 Characteristics of the analytical method derived from the standard calibration curve

Compound	Linearity range (µg/ml)	Correlation coefficient	Slope	Intercept
Maltose	5-25	0.999	154.16	112.56

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The chromatographic analysis of aqueous leaf extracts from plants was performed using a mobile phase consisting of acetone and aqueous 82:18 (V/V) solutions. The flow rate used was 1 ml per minute. But Tulsi and Mulhethi both do not show peak or retention time for maltose as compared to maltose standard reference with a retention time of 15.67 minutes. However, the results indicated that the peaks of various plant extracts did not significantly differ from the standard quercetin, demonstrating maltose absence in both species. This study highlighted the differences in chemical constituents between the two plants.

Conclusion:

In conclusion, the process of extracting and analyzing the aqueous extracts of Tulsi and Mulethi has shed important light on the chemical make-up and possible medical uses of these plants. With an emphasis on quercetin as a marker component, the identification and quantification of certain bioactive chemicals were made possible through the use of high-performance liquid chromatography (HPLC). It was shown that quercetin exists in both plant extracts as a secondary metabolite, underlining its potential role in the observed therapeutic activities. The extracts further separated the chemical components of these plants due to the absence of maltose. These discoveries advance our knowledge of the bioactive substances found in tulsi and mulethi and lay the groundwork for future investigations into their pharmacological effects and therapeutic potential. Overall, this research supports the conventional usage of these plants in herbal medicine and adds to the body of knowledge in the field of phytochemical analysis.

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