

GC-MS Analysis of Phytochemicals in aqueous extract of Tulsi and Mulhethi

Sachin Kumar¹, Harsh Dhankhar² and Seema Kumari*

Department of Botany, Baba Mastnath University, Asthal Bohar, Rohtak, Haryana, India

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ABSTRACT

Extraction process specifically targets isolation of carbohydrates, glycosides, and secondary metabolites from leaves. These compounds play crucial roles in Mulethi and Tulsi's biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties. By extracting and analysing these components, a deeper understanding of these plants' medicinal potential can be gained. The identification of the extracted compounds was done using various chromatographic techniques. Chromatography is a powerful tool that separates and characterizes complex mixtures. Techniques such as gas chromatography (GC) were employed to separate the individual components of the extract and determine their chemical structures. The aqueous extract was screened and qualitatively analysed to detect specific compounds. The analytical technique combines GC separation capabilities with MS detection and identification capabilities. It provides valuable information about the chemical composition of the extracts, allowing the identification of individual compounds present in the samples. These compounds are known for their pharmacological activities and are often responsible for plants' medicinal properties.

Keywords: Tulsi, Mulhethi, GC-MS, Phytochemicals.

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). World Health Organization (WHO) estimates that almost 80% of the world's population relies mostly on traditional plant-based medicines to meet their most basic healthcare needs (WHO: Geneva; 2000). Traditionally, Tulsi and Mulethi have been used as a whole herb to treat a good number of diseases. The wide range and diversities in the action of these two herbs can be a result of the synergistic effect of its phytochemical components. Due to the medicinal, nutritional and spiritual properties, Tulsi can be found in every house of Indian Subcontinent. It is usually also

known as "Holy Basil or Sacred Basil" (Badore *et al.*, 2020). A recent study by Lopresti *et al.*, (2022) concluded that supplementation with extract of tulsi lowers stress and improves sleep quality.

Mulhethi, also known as *Yashtimadhu*, is a traditional plant which is widely used in Ayurveda. It is used to treat a variety of ailments including cough, asthma, bronchitis, diabetes, gastric ulcers, skin problems, inflammation, anaemia, premature greying of hair and others. Clinical and experimental research suggests that it possesses anti-inflammatory, antiviral, Immunomodulatory, Hepatoprotective, antimicrobial, antioxidant, anti-cancer, and cardio protective activities (Chandran *et al.*, 2022).

The present study deals with phytochemical analysis, extraction of specific compounds, and

chromatographic techniques provide valuable insights into the chemical profile and potential therapeutic benefits of Mulethi and Tulsi leaves.

Methodology

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, Sonapat 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 washing in distilled water, 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. The preparation process was carefully monitored to ensure that all the ingredients were used in the exact proportions specified in the recipe. Finally, the extract was ready to be used in the product.

Plant extracts preparation

To prepare the aqueous solvent extraction from Mulethi and Tulsi leaves, the Soxhlet apparatus was employed. The leaves were first dried and ground into a fine powder. This powder was then placed in a thimble, which was inserted into the Soxhlet extraction chamber. The extraction process involved the circulation of a suitable solvent, such as water, through the powdered leaves. The solvent repeatedly passed through the extraction chamber, ensuring efficient extraction of the desired compounds

from the plant material. The extracted solution was collected, carefully labelled, and stored for future experimental use. 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.

Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical constituents of essential oils from the fresh and dried leaves of Tulsi and Mulethi were analyzed by GC/MS. The analysis was performed using a ThermoQuest instrument coupled with a mass spectrometer. The same column and temperature programming were used in the GC analysis, conducted by the technical staff of Dr. A.P.J Abdul Kalam-CIL Lab at Guru Jambheshwar University of Science and Technology, Hisar. Identification of the different components present in the essential oils was accomplished by comparing the mass spectral data obtained with those available in spectral libraries such as NIST, Wiley GC-MS, and Duke's database. This comparative analysis allowed the identification of specific compounds based on their mass spectra.

By employing these analytical techniques and referencing the available databases, the chemical constituents present in Tulsi and Mulethi essential oils were successfully characterized and recognized. The fresh leaves of Tulsi (*Ocimum sanctum*) contain several major constituents, including eugenol (20.79 RT), β -caryophyllene (22.19 RT), Tetracontane, 3,5,24-trimethyl (24.24 RT), Octatriacontylpentafluoropropionate (26.45 RT) and Sulfurous acid, butyl Octadecyl ester (38.16 RT). These five compounds collectively make up approximately 93.23 ± 6.24 percent of the total identified compounds. Among these constituents, eugenol is the predominant compound, comprising more than half of the total identified compounds. A table provides a comprehensive list of chemical compounds present in Tulsi fresh leaves (Figure 1).

The GC-MS analysis of Mulethi leaves aqueous extract showed a molecular ion peak at 413 m/z. Mulethi leaves were analyzed using the Duke's database, and it was found that the major constituent present in the leaves is prunetin (5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one).

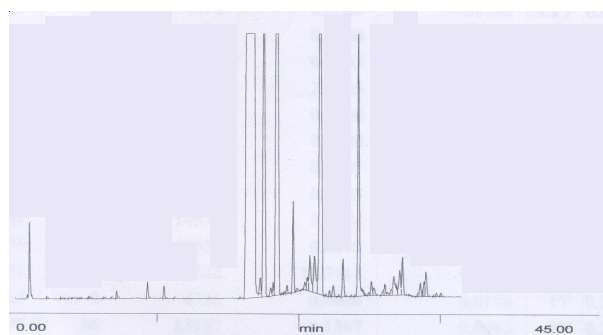


Fig. 1. GC-MS analysis of Tulsi leaves aqueous extract

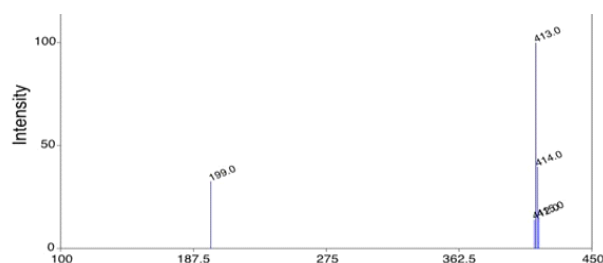


Fig. 2. GC-MS analysis of Mulhethi leaves aqueous extract

Prunetin is an allosteric inhibitor of human liver aldehyde dehydrogenase. This enzyme plays a role in the metabolism of alcohol and other toxic compounds in the liver. By inhibiting this enzyme, prunetin may have potential therapeutic applications in the treatment of certain liver disorders. Furthermore, prunetin has been studied for its effects on blood pressure regulation. It lowers blood pressure in spontaneously hypertensive rats, suggesting its potential as an antihypertensive agent.

Conclusion

From this current study, we concluded that the aqueous extract of leaves *Ocimum sanctum* and *Glycyrrhiza glabra* have phytochemical. The medicinal property of these herbs may be because of the presence of the active biochemical's and phytochemical. This study concluded that the plants are reservoir of essential natural antioxidant and may be

beneficial in oxidative stress protection. The antioxidant nature of plants is due to the occurrence of secondary metabolites. The current study concluded that different solvent extracts could be seen as a better source of rich phytochemical compounds. Prunetin relaxes isolated rat aortic rings, which are models for studying vascular smooth muscle function. This relaxation effect is believed to occur through the blocking of calcium channels in smooth muscle cells, leading to vasodilation and improved blood flow. Eugenol is active constituent present in tulsi, has been found to be largely responsible for the therapeutic potentials. Although because of its great therapeutic potentials and wide occurrence in India the practitioners of traditional systems of medicine have been using tulsi for curing various ailments, a rational approach to this traditional medical practice with modern system of medicine is, however, not much available.

References

- Badore, A., Pandit, P., Vijay Shree and Nilosey, V.S. 2020. Comparative Phytochemical Studies of *Ocimum sanctum* and *Mentha Arvensis* Ethanolic Leaf Extract. *International Journal for Research Trends and Innovation, (IJRTI)*. 5(12): ISSN:2456-3315.
- Chandran, A., Syam, R.J., Jerone, J.J. and V, S.K. 2022. Ethnopharmacological study about *Glycyrrhiza glabra* L. (Licorice) based on Ayurveda, An Indian System of Traditional Medicine- A Review. *International Journal of Ayurvedic Medicine*. 13(3): 587-600. <https://doi.org/10.47552/ijam.v13i3.2867>
- Lopresti, A.L., Smith, S.J., Metse, A.P. and Drummond, P.D. 2022. A randomized, double-blind, placebo-controlled trial investigating the effects of an *Ocimum tenuiflorum* (Holy Basil) extract (Holixer TM) on stress, mood, and sleep in adults experiencing stress. *Front Nutr*. 9: 965130.
- Nikhil Sudhakar Gulhane, Chaitanya D Ghode, Amol G Jadhao and Prashant A Patil, 2021. Study of medicinal uses of *Ocimum sanctum* (Tulsi). *J Pharmacogn Phytochem*. 10(2): 1427-1431.
- WHO, 2000. Integration of traditional and complimentary medicine Into National Healthcare systems. *J. Manipulative & Physiological Therapeutics*. 139 : 140.