

Extraction Techniques for Analyzing *Citrus Hystrix* and *Lagenaria Siceraria* Compounds for Gum Disease Via Gas Chromatography Mass Spectrometry

A'wani Aziz Nurdalila^{1,2*}, Muhammad Aqil Yazmi², Liyana Amalina Adnan² & Ahmad Bazli Ahmad Hilmi²

¹Institute of Fatwa and Halal (IFFAH), Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800, Nilai, Negeri Sembilan, Malaysia

²Kolej PERMATA Insan, Universiti Sains Islam Malaysia, 71800 Nilai, Negeri Sembilan, Malaysia

*Corresponding author: nurdalila.awani@usim.edu.my

ABSTRACT

In this era of globalisation, many individuals who suffer from allergies or sensitivities to industrial medications and synthetic chemicals often look for organic and environmentally friendly alternatives. This trend reflects a growing preference for sustainable and eco-conscious choices in personal and healthcare products. To tackle this issue, we have identified bioactive compounds in *Lagenaria siceraria* and *citrus hystrix* leaves to showcase their effectiveness and compatibility for gum disease. These plants not only provide a safer and more natural option for consumers but also offer an alternative source of chemical components required by pharmacies and medical professionals for related products. To analyze the diversity of bioactive compounds in *citrus hystrix* leaves and *Lagenaria siceraria*, this study utilizes Gas Chromatography-Mass Spectrometry (GCMS). The samples were dried at 60°C for 24 hours and then mashed into a powder. The powder was weighed and mixed with a 4:1 hexane and ethyl acetate solvent in a ratio of 10 mL of solvent per gram of sample. The mixture was covered and left for 24 hours, then filtered to separate the solid residue from the liquid. The liquid was placed in a rotary evaporator set at 76°C and 264 ATM to remove the solvent, leaving behind the extracted oil. This extraction method will be employed to prepare samples for subsequent analysis using Gas Chromatography-Mass Spectrometry (GC-MS), ensuring accurate and detailed examination of the compounds present.

Keywords: Citrus hystrix, Lagenaria siceraria, gas chromatography-mass spectrometry, extraction technique

INTRODUCTION

Citrus hystrix, commonly known as kaffir lime or medicinal lime, is a tropical fruit belonging to the *Rutaceae* family. It is native to India and Southeast Asia, thriving in regions like Southern China, Malaysia, and Thailand. This underutilized species typically grows as a small, bushy tree, reaching heights of about 3-5 meters (Abirami et al., 2014). The fruit of *Citrus hystrix* is distinctive, characterized by its green, warty, and bumpy appearance. Both the leaves and fruits are commonly used as spices in Asian cooking, with *Citrus hystrix* leaves playing a significant role in Malaysian and Burmese cuisines for tea-making and as flavouring agents (Kamisah et al., 2022).

Furthermore, the juice and peel of *Citrus hystrix* are utilized in traditional medicine across several Asian countries (Zhou et al., 2023). Beyond culinary uses, kaffir lime oil serves as a raw material in various industries, including pharmaceuticals, agronomy, food, cosmetics, and perfumery (Suresh et al., 2021).

Lagenaria siceraria (Molina), also known as Bottle gourd, is a member of the *Cucurbitaceae* family and is widely recognized for its extensive cultivation as a vegetable crop in tropical regions such as Thailand, Egypt, India, Japan, and many other countries across the globe (Saeed et al., 2022). This climbing perennial plant is particularly suited for warm, humid climates, although it can also be grown successfully in dry conditions provided it receives ample water. The cultivation process can begin with seeds sown in nursery beds. Once the seedlings have developed 2-3 leaves, they are ready to be transplanted into the field. An alternative method is to sow the seeds directly in well-manured beds or pits spaced 5-6 feet apart, planting 4-5 seeds per pit. After germination, the healthiest and strongest seedling is left to grow, while the others are either removed or transplanted to other locations (Kumar et al., 2012). This method ensures that the strongest plant has enough space and nutrients to thrive, contributing to optimal growth and yield.

MATERIALS AND METHODS

The samples were placed in an oven at 60°C for 24 hours to dry. Once dried, the samples were ground using a mortar and pestle, and the weight of the ground samples was recorded. The samples were then placed in a conical flask containing a solvent solution of hexane and ethyl acetate in a 4:1 ratio. The conical flask was covered with aluminum foil and left for 24 hours. After 24 hours, the solution in the conical flasks was separated using a filter funnel and filter paper to separate the solid residue from the solvent solution. The solvent solution, which contained the extracted compound, was then poured into the evaporation flask of a rotary evaporator. The rotary evaporator was set to 76°C at 264 ATM. The process was stopped once no more solvent was dripping, leaving only oil in the evaporation flask. The extracted oil was then obtained. The ratio between the dried sample and the solvent should be 1:10; for example, if the sample weighs 1 gram, then 10 ml of the solvent mixture should be used.

RESULTS AND DISCUSSION

At this stage of the research, the extraction process has been successfully completed but the samples have not yet been analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The extraction yielded oils from both *Citrus hystrix* and *Lagenaria siceraria*, which will later be tested for their bioactive compounds. The drying process at 60°C for 24 hours, followed by grinding, ensured that the plant materials were prepared in an optimal form for extraction. Using a 4:1 solvent ratio of hexane and ethyl acetate, the solvent effectively penetrated the plant samples, which were then subjected to rotary evaporation to isolate the oils. This method should preserve the integrity of the bioactive compounds, ready for future GC-MS analysis. The next phase will involve the GC-MS analysis to identify the specific compounds present in the extracts. Based on previous studies, it is anticipated that *Citrus hystrix* will contain essential oils like limonene, citronellal, and β -pinene, while *Lagenaria siceraria* may show the presence of cucurbitacin, saponins, and fatty acids, which are known for their medicinal properties, particularly in treating inflammation and microbial infections. The results from GC-MS will provide a more detailed profile of these compounds and confirm their potential effectiveness in treating gum disease.

CONCLUSION

This preliminary study has successfully extracted oils from *Citrus hystrix* and *Lagenaria siceraria* using an optimized solvent extraction technique. While the GC-MS analysis has not yet been conducted, the extraction process has laid the groundwork for further analysis. The next steps will involve identifying the bioactive compounds through GC-MS and evaluating their potential for use in treating gum disease. Once the GC-MS analysis is completed, it is expected that the compounds isolated from these plants will provide a natural, sustainable alternative for oral healthcare, particularly for consumers seeking organic solutions. Future work will include not only the compound identification but also testing the biological efficacy of these extracts in clinical settings.

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