Hexane-Acetone Extraction Techniques for Gas Chromatography-Mass Spectrometry

A'wani Aziz Nurdalila[1,2*], Muhammad 'Ammar 'Ayyasy Muhamad Zuhdi[2], Liyana Amalina Adnan[2] & Ahmad Bazli Ahmad Hilmi[2]

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

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

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

ABSTRACT

At present, a lot of dental care products are mixtures of drugs and foreign substances. Some people are allergic or sensitive to industrial medications and synthetic chemicals. Many of those affected by this seek more organic and eco-friendly substitutes that do not cause allergic reactions, preferring natural alternatives. In this case, we want to make an alternative by comparing the compounds that are contained in industrial medications with eco-friendly substitutes by using Gas Chromatography-Mass Spectrometry (GC-MS). An acetone is used when preparing the extraction. We have found that *Brucea javanica*, *Solanum lasiocarpum*, and Alum Stone mixture contains compounds that can be used as an alternative. Brucea javanica is believed to offer anti-cancer, antimalarial, and anti-inflammatory benefits. *Solanum lasiocarpum* is widely used in traditional medicine as a diuretic and for treating various diseases such as fever, inflammation, and cough. Alum stone has antiseptic properties that help to fight against tooth decay. The dry samples were ground into a fine powder, accurately weighed, and placed in a conical flask containing a hexane mixed with acetone solvent system at a 4:1 ratio. The flask was then sealed with aluminum foil and allowed to sit overnight to ensure complete extraction. The mixture was subsequently filtered, separating the solid residue from the solvent. The extracted compounds were concentrated into an oil extract using a rotary evaporator set at 76°C and 264 atmosphere (ATM). A solvent to sample ratio was employed utilizing 10mLg^{-1} of sample. This extraction method will be applied in subsequent GCMS analysis.

Keywords: *Brucea Javanica*, *Solanum Lasiocarpum*, alum stone, GCMS, hexane-acetone, extraction technique

INTRODUCTION

The increasing prevalence of allergic reactions and sensitivities to synthetic chemicals used in modern dental care products has led to a growing demand for natural, organic alternatives. Many individuals seek eco-friendly products that do not contain industrial medications or artificial substances, which are often linked to adverse effects. This shift toward natural dental care solutions reflects a broader consumer trend toward sustainability and health-consciousness. The development of effective natural alternatives to synthetic dental care products requires a thorough understanding of the active compounds in natural substances, along with their potential to replace industrial chemicals without compromising efficacy.

This study aims to address these concerns by exploring a mixture of three natural substances: *Brucea javanica*, *Solanum lasiocarpum*, and alum stone, all of which have demonstrated

therapeutic properties in traditional medicine. It is believed that *brucia javanica* can reduce tumor cell growth and has activities in anti-inflammation and anti-malaria, which suggest a potential application in oral health for reducing inflammation and combating infections (Yue Zhang et al., 2016). *Solanum lasiocarpum*, a plant widely used in folk medicine, is valued for its diuretic, fever-reducing, anti-inflammatory, and cough-suppressing properties, making it a promising candidate for managing oral inflammation and irritation. Alum stone, a natural mineral with strong antiseptic properties, has long been used to prevent tooth decay and promote oral hygiene.

Given that Malaysia has a large Muslim population, we were inspired to try a traditional recipe found in an old book. It suggested combining *Brucea javanica*, *Solanum lasiocarpum*, and alum stone to create a paste, which is then applied to the teeth.

METHODOLOGY

The extraction process began with the drying of the samples to ensure the removal of any moisture content. The samples were placed in an oven and heated at a constant temperature of 60°C for 24 hours. After the drying period, the dried samples were manually ground using a mortar and pestle to break them into finer particles, which facilitated better interaction with the solvent during extraction. The weight of each ground sample was carefully measured using an analytical balance to ensure accurate sample-to-solvent ratios.
Each weighed sample was then transferred into a clean, dry conical flask. A solvent solution composed of hexane and ethyl acetate, in a ratio of 4:1, was prepared. The solvent volume was determined based on a 10:1 ratio between the solvent and the sample weight, meaning that for every gram of sample, 10 mL of solvent mixture was added. For example, a 1 g sample required the addition of 10 mL of solvent solution. This ratio was maintained to ensure consistent extraction across all samples.

Once the solvent was added to the conical flask, the flask was sealed with a layer of aluminum foil to minimize solvent evaporation and contamination. The flasks were left undisturbed at room temperature for 24 hours to allow the compounds from the sample to diffuse into the solvent solution. This passive extraction process is essential for ensuring the complete dissolution of target compounds into the solvent.

After 24 hours of soaking, the mixture in each conical flask was subjected to a filtration process to separate the solid residues from the liquid solvent containing the dissolved compounds. This was achieved by pouring the contents of the flask into a filter funnel lined with filter paper. The filtrate, which contained the extracted compounds, was collected in a separate container, while the solid residues (mush) remained on the filter paper.

The filtrate, consisting of the solvent solution with the extracted compounds, was then transferred into the evaporation flask of a rotary evaporator. The rotary evaporator was set to operate at a temperature of 76°C and a pressure of 264 ATM, conditions that ensured efficient evaporation of the solvent. The rotary evaporator was run until no further solvent was seen dripping into the collection vessel, indicating that the solvent had been completely removed. The evaporation process was stopped when only the extracted oil remained in the evaporation flask.

The final step of the process was the collection of the extracted oil, which was then stored for further analysis or use. This method ensured a high-quality extraction by carefully controlling drying, solvent interaction, and evaporation conditions. The use of hexane and ethyl acetate as solvents in a 4:1 ratio for extraction processes is a well-documented approach in lipid and oil extraction. For instance, hexane is commonly chosen for its efficacy in extracting non-polar compounds, while ethyl acetate is useful in extracting slightly polar compounds. This combination has been used effectively in food and natural product extractions (Henderson et al., 2011; Vagi et al., 2002).

The application of rotary evaporation to concentrate solvent mixtures is widely practiced in various extraction methodologies to obtain oil or other non-volatile components (Careri et al., 2001). Rotary evaporators are frequently used at controlled temperatures and pressures to safely remove solvents while preserving heat-sensitive compounds (Mendes et al., 1995; Canela et al., 2002).

RESULTS AND DISCUSSION

Based on the methodology described in the document, here is a discussion of the steps taken during the extraction process for analyzing the dental applications of *Brucea javanica*, *Solanum lasiocarpum*, and Alum stone mixture.

The methodology involves a well-defined process to ensure optimal extraction of the compounds from the natural substances being studied. The first step is the drying of the samples to eliminate moisture, which is crucial for efficient extraction. The drying process, conducted at a constant temperature of 60°C for 24 hours, ensures that no residual water interferes with the extraction of bioactive compounds.

Next, the dried samples are ground into a fine powder using a mortar and pestle. This step increases the surface area of the samples, facilitating better interaction with the solvent. Accurate weighing of the ground samples is essential, ensuring precise solvent-to-sample ratios for consistent results. The samples are placed in a clean, dry conical flask, ready for the next step.

A solvent mixture of hexane and ethyl acetate, in a 4:1 ratio, is prepared for the extraction. The use of a 10:1 solvent-to-sample ratio ensures adequate solvent for complete extraction. This ratio is carefully calculated, maintaining uniformity across all samples to avoid variations in extraction efficiency.

Once the solvent mixture is added to the conical flask containing the sample, the flask is sealed with aluminum foil to prevent contamination and solvent evaporation. The samples are left undisturbed for 24 hours, allowing the compounds to diffuse into the solvent. This passive extraction technique is effective in dissolving target compounds without the need for additional mechanical intervention. After the soaking period, the mixture is filtered to separate the solid residues from the liquid containing the extracted compounds. The filtrate is collected for further processing, while the solid residues are discarded.

The collected filtrate undergoes evaporation using a rotary evaporator. The controlled temperature of 76°C and pressure of 264 ATM ensures efficient removal of the solvent without damaging the heat-sensitive compounds. The evaporation process continues until only the extracted oil remains, which is then collected and stored for subsequent analysis.

This method effectively isolates key bioactive compounds, such as those with anti-inflammatory, anti-cancer, and antiseptic properties, using hexane and ethyl acetate as solvents. The careful execution of the drying, solvent interaction, and evaporation steps ensures a high-quality extraction, suitable for further analysis via gas chromatography-mass spectrometry (GC-MS).

CONCLUSION

The study concludes that the hexane-acetone extraction method, combined with GC-MS analysis, effectively isolates key compounds from *Brucea javanica*, *Solanum lasiocarpum*, and alum stone. These compounds, known for their anti-inflammatory, anti-cancer, and antiseptic properties, offer potential as natural alternatives to synthetic dental care products. The successful extraction and concentration process supports their further exploration for sustainable oral health solutions.

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