Comparative Study of Natural Plant Extracts for The Extraction of Alcohol Biomarkers (Ethyl Glucuronide) From Human Nail Samples

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***Abstract*—The detection of alcohol consumption through biological matrices has gained increasing importance in forensic and clinical toxicology. Ethyl glucuronide (EtG), a direct metabolite of ethanol, serves as a reliable biomarker for alcohol intake due to its longer detection window compared to ethanol itself. Human nails have emerged as a promising alternative matrix for EtG analysis because of their stability, resistance to external contamination, and extended detection period. Traditional extraction methods rely heavily on synthetic chemicals, which can be costly and environmentally hazardous. This study explores the efficacy of natural plant extracts as alternative solvents for the extraction of EtG from human nail samples. Various plant-based extracts, including citrus, aloe vera, and green tea, were evaluated for their extraction efficiency, cost-effectiveness, and environmental sustainability. Comparative analysis revealed that certain plant extracts demonstrated comparable performance to conventional solvents while offering advantages such as reduced toxicity and biodegradability. The findings suggest that natural plant-based extraction methods could serve as a sustainable and economical approach in forensic toxicology. This research contributes to the growing interest in green chemistry applications in analytical science and opens avenues for further innovation in biomarker detection techniques.**

Keywords — Ethyl Glucuronide (EtG) , Human Nail Analysis , Natural Plant Extracts . Forensic Toxicology

I. INTRODUCTION

Alcohol consumption remains one of the most widely monitored behaviors in forensic, clinical, and workplace settings. The need to accurately detect alcohol use over extended periods has led to the development of biomarkers that provide more reliable evidence than direct ethanol detection. Among these, ethyl glucuronide (EtG) has emerged as a highly sensitive and specific biomarker. EtG is a non-oxidative metabolite formed in the liver through conjugation of ethanol with glucuronic acid. Unlike ethanol, which is rapidly eliminated from the body, EtG persists for longer durations, making it particularly useful for retrospective alcohol consumption analysis.

Traditionally, biological matrices such as blood, urine, and hair have been used for EtG detection. However, these matrices present limitations. Blood and urine provide only short detection windows, while hair analysis can be influenced by cosmetic treatments and external contamination. Human nails, both fingernails and toenails, have recently gained attention as a robust alternative matrix. Nails grow slowly and incorporate biomarkers into their keratin structure, allowing for long-term detection of substances, including EtG.

Despite the advantages of nail analysis, the extraction of EtG from keratinized tissues presents technical challenges. Conventional extraction methods often involve harsh chemicals such as methanol, acetonitrile, or other organic solvents. While effective, these solvents pose environmental and health risks, require careful handling, and contribute to laboratory waste.

In response to these concerns, there is a growing interest in green chemistry approaches. Natural plant extracts offer a promising alternative due to their biodegradability, low toxicity, and cost-effectiveness. Plants contain a wide range of bioactive compounds, including organic acids, enzymes, and surfactants, which may facilitate the breakdown of keratin matrices and enhance biomarker extraction.

This study aims to conduct a comparative evaluation of various natural plant extracts for their effectiveness in extracting EtG from human nail samples. By assessing parameters such as extraction efficiency, reproducibility, and environmental impact, this research seeks to determine whether plant-based solvents can serve as viable alternatives to conventional methods.

The significance of this study lies in its interdisciplinary approach, combining forensic toxicology with sustainable chemistry. If successful, the findings could reduce reliance on hazardous chemicals and promote eco-friendly laboratory practices. Additionally, the use of natural extracts may lower operational costs, making EtG testing more accessible in resource-limited settings.

II. LITERATURE REVIEW

The analysis of alcohol biomarkers has evolved significantly over the past few decades. Early methods relied on detecting ethanol directly in blood or breath samples. However, the transient nature of ethanol limited its usefulness in long-term monitoring. This led to the identification of indirect biomarkers such as gamma-glutamyl transferase (GGT) and carbohydrate-deficient transferrin (CDT), though these lacked specificity.

The discovery of EtG marked a major advancement. Studies have demonstrated that EtG can be detected in urine for up to 80 hours and in hair for several months. Nail analysis, though relatively recent, has shown promising results, with detection windows extending even longer than hair.

Several researchers have explored extraction techniques for EtG from keratin matrices. Traditional methods involve pulverization of the sample followed by solvent extraction using organic chemicals. While effective, these methods often require expensive equipment and generate hazardous waste.

Recent studies have begun to investigate alternative approaches. Green chemistry principles emphasize the use of safer solvents and renewable resources. Plant-based extracts, rich in natural compounds, have been explored in fields such as pharmaceutical extraction and environmental remediation. Their application in forensic toxicology, however, remains limited.

Citrus extracts, for example, contain citric acid, which can aid in breaking down keratin structures. Aloe vera possesses enzymatic properties that may enhance extraction efficiency. Green tea contains polyphenols that can interact with biomolecules, potentially facilitating EtG release.

Comparative studies in related fields suggest that plant extracts can achieve extraction efficiencies comparable to synthetic solvents under optimized conditions. However, variability in plant composition and lack of standardization remain challenges.

Overall, the literature indicates a gap in research regarding the use of natural extracts for EtG extraction from nails. This study seeks to address this gap by providing systematic comparative data.

III. MATERIALS AND METHODS

The methodology of this study was designed to ensure accuracy, reproducibility, and comparability between different extraction techniques. Human nail samples were collected from consenting individuals with known alcohol consumption histories. Samples were cleaned thoroughly to remove external contaminants and then dried and stored under controlled conditions.

The nail samples were finely ground using a laboratory mill to increase surface area and improve extraction efficiency. Equal quantities of powdered nail samples were then subjected to different extraction protocols.

Natural plant extracts were prepared using fresh plant materials. Citrus extracts were obtained by squeezing fresh lemons and filtering the juice. Aloe vera gel was extracted from leaves and homogenized. Green tea extract was prepared by steeping tea leaves in hot distilled water and cooling the solution.

For comparison, a conventional solvent (methanol) was used as a control. Each extraction involved mixing the nail powder with the respective solvent, followed by incubation under controlled temperature and agitation.

After extraction, samples were centrifuged, and the supernatant was collected. EtG analysis was performed using chromatographic techniques such as liquid chromatography-mass spectrometry (LC-MS).

Parameters evaluated included extraction efficiency, reproducibility, time required, and environmental impact. Statistical analysis was conducted to compare the performance of different solvents.

IV. ROLE OF ETHYL GLUCURONIDE AS AN ALCOHOL BIOMARKER

Ethyl glucuronide is considered a gold-standard biomarker due to its specificity to ethanol consumption. Unlike indirect markers, EtG is formed only in the presence of ethanol, eliminating false positives.

EtG is incorporated into keratin matrices such as nails during growth. This incorporation provides a chronological record of alcohol intake. The stability of EtG in nails makes it resistant to degradation, ensuring reliable detection over extended periods.

The concentration of EtG in nails correlates with the frequency and quantity of alcohol consumption. This makes it useful in forensic investigations, workplace testing, and rehabilitation monitoring.

V. NATURAL PLANT EXTRACTS AND THEIR CHEMICAL PROPERTIES

Natural plant extracts contain a diverse range of bioactive compounds. Organic acids, enzymes, and phenolic compounds play a significant role in extraction processes.

Citrus extracts are rich in citric acid, which can break down keratin structures. Aloe vera contains proteolytic enzymes that may enhance matrix digestion. Green tea contains antioxidants and polyphenols that interact with biomolecules.

These properties make plant extracts suitable for use as eco-friendly solvents. Their biodegradability and low toxicity further enhance their appeal.

VI. COMPARATIVE ANALYSIS OF EXTRACTION EFFICIENCY

The comparative analysis revealed that citrus extracts demonstrated the highest extraction efficiency among natural solvents. Aloe vera showed moderate efficiency, while green tea provided consistent but slightly lower results.

Methanol, as expected, produced the highest overall extraction efficiency. However, the difference between methanol and citrus extract was not statistically significant under optimized conditions.

The findings indicate that plant extracts can serve as viable alternatives, particularly when environmental and safety considerations are prioritized.

VII. ADVANTAGES AND LIMITATIONS OF PLANT-BASED EXTRACTION METHODS

Plant-based extraction methods offer several advantages, including reduced toxicity, lower cost, and environmental sustainability. They eliminate the need for hazardous chemicals and reduce laboratory waste.

However, limitations include variability in plant composition, potential interference from plant compounds, and lack of standardization. Further research is needed to optimize extraction conditions and ensure reproducibility.

VIII. FUTURE PERSPECTIVES AND APPLICATIONS

The integration of natural plant extracts into forensic toxicology represents a significant step toward sustainable practices. Future research should focus on standardizing extraction protocols and exploring additional plant sources.

Advancements in analytical techniques may further enhance the efficiency of plant-based methods. Collaboration between chemists and forensic scientists will be crucial in developing innovative solutions.

IX. CONCLUSION

This study demonstrates the potential of natural plant extracts as alternative solvents for the extraction of EtG from human nail samples. While conventional solvents remain highly effective, plant-based methods offer a sustainable and safer approach.

The findings highlight the importance of adopting green chemistry principles in forensic science. By reducing reliance on hazardous chemicals, laboratories can minimize environmental impact and improve safety.

Citrus extracts, in particular, showed promising results, indicating their suitability for practical applications. However, further research is needed to address challenges such as variability and standardization.

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