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The Role of Herbal Anticoagulants in Liquid Biopsy Applications for Oral Squamous Cell Carcinoma

Srivarsha Ranjeet[1], Monal Yuwanati[2*], Senthil Murugan Mullainathan[3]

[1]Department of General Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai600077, Tamilnadu, India.
[2]Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai600077, Tamilnadu, India.
[3]Department of Oral Oncology & Reconstructive Microsurgery, Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai600077, Tamilnadu, India.

***E-mail** ✉ monalyuwanti.sdc@saveetha.com

Abstract

Oral squamous cell carcinoma (OSCC), responsible for the vast majority of head and neck cancers (90–96%), remains a major global health concern. Molecular-level insight into this disease is critical for developing targeted therapies and enhancing patient care. This study explores the potential of *Gymnema sylvestre* (*G. sylvestre*), a natural compound, as an alternative anticoagulant to ethylenediaminetetraacetic acid (EDTA) for preserving blood samples intended for circulating tumor DNA (ctDNA) extraction. In this prospective analysis, blood was drawn from 20 OSCC patients and stored in tubes containing either *G. sylvestre* or EDTA. ctDNA levels were assessed using polymerase chain reaction (PCR). The results showed that both *G. sylvestre* and EDTA performed comparably in preserving ctDNA yield. These findings suggest that G. sylvestre could serve as a viable, plant-based substitute for traditional chemical anticoagulants in the molecular profiling of OSCC.

Keywords: Gymnema sylvestre, EDTA, ctDNA, Oral cancer, Natural anticoagulant, OSCC

Introduction

Oral squamous cell carcinoma (OSCC) represents the predominant form of head and neck malignancies, comprising approximately 90–96% of all such cancers [1]. Despite advancements in clinical management—including surgical intervention, chemotherapy, and radiotherapy—OSCC continues to be associated with poor survival outcomes. The five-year survival rate for OSCC has stagnated over the past decades, ranging between 40% and 53.7% [2]. Prognosis further declines in cases of metastasis, recurrence, or advanced-stage presentation. Moreover, factors related to the disease's origin and development—its etiopathogenesis—play a critical role in determining disease progression and treatment response.

Timely diagnosis remains one of the most effective strategies for improving survival and reducing disease burden in OSCC patients [3]. Current diagnostic modalities include clinical examination and imaging techniques, but confirmation typically requires a histopathological biopsy. Unfortunately, this method is invasive and often met with patient reluctance, especially during the early stages when symptoms are subtle or ambiguous. Consequently, diagnosis is frequently delayed until the disease has progressed significantly, limiting treatment options to palliative care. Furthermore, OSCC treatment responses vary greatly between individuals, partly due to an incomplete understanding of the tumor's underlying molecular mechanisms.

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To gain insight into OSCC pathogenesis, researchers have turned to molecular tools [4]. Carcinogenesis involves genetic mutations and abnormalities, including alterations in complementary DNA (cDNA) [5]. By identifying these mutations, researchers hope to pave the way for targeted therapies that move beyond conventional treatment protocols [6]. At present, OSCC treatment largely follows a uniform approach, which may not be effective for all patients due to tumor heterogeneity. This has driven growing interest in personalized medicine tailored to each individual's unique genetic profile.

Molecular research relies on biological specimens such as tissues and bodily fluids. While tissue samples offer direct insight, obtaining them can be invasive and unsuitable for early detection. In contrast, liquid biopsy methods—especially those analyzing circulating tumor DNA (ctDNA)—offer a minimally invasive alternative with promising diagnostic potential [7]. However, the integrity and quantity of ctDNA can be influenced by how samples are processed, stored, and transported [8].

Ethylenediaminetetraacetic acid (EDTA) is the standard anticoagulant used for collecting blood samples for ctDNA analysis. While widely adopted, EDTA has been found to fragment ctDNA, potentially reducing the accuracy of molecular testing. Moreover, synthetic anticoagulants like EDTA may interact with genetic materials, limiting their suitability for sensitive assays [9]. Recently, attention has shifted toward natural alternatives that may offer fewer side effects on nucleic acid integrity.

Gymnema sylvestre (*G. sylvestre*), a medicinal plant known for its therapeutic properties, has demonstrated anticoagulant activity in biological studies [10, 11]. Given its natural origin and biological compatibility, *G. sylvestre* has been proposed as a potential alternative to synthetic anticoagulants like EDTA. However, its impact on ctDNA preservation remains unclear. This study aims to compare the ctDNA yield from blood samples stored in *G. sylvestre* extract to those preserved with EDTA, thereby evaluating the feasibility of *G. sylvestre* as a natural anticoagulant in molecular diagnostics for OSCC.

Materials and Methods

This prospective study was conducted on patients diagnosed with oral squamous cell carcinoma (OSCC) who presented for oncology care at the hospital. Before sample collection, informed consent was obtained from all participants. Ethical approval for the study was granted by the Institutional Human Ethics Committee (Approval no. IHEC/SDC/GPATH-2000/22/101).

Sample collection

Peripheral blood (2 ml) was collected from each patient under aseptic conditions. The blood was then divided equally and transferred into two separate collection tubes—one containing *G. sylvestre* extract and the other with ethylenediaminetetraacetic acid (EDTA) as anticoagulants. Following collection, samples were centrifuged at 12,500 rpm for 15 minutes to separate the plasma. The obtained plasma was stored at –80 °C until further use.

DNA extraction from plasma was carried out using a commercially available DNA extraction kit, according to the manufacturer's protocol. The extracted DNA (100 µL) was then diluted with 70 µL of buffer AE and preserved at –20 °C for downstream applications.

ctDNA quantification

To isolate ctDNA, a 100 µL aliquot of each plasma sample was treated with 1 mL of Trizol reagent and homogenized thoroughly. Subsequently, 0.2 mL of chloroform was added to each tube, followed by brief vortexing. The mixture was centrifuged at 12,500 rpm for 15 minutes, and the resulting aqueous phase was carefully transferred into a fresh tube. An equal volume of isopropyl alcohol was added to precipitate the nucleic acids, followed by centrifugation at 8,500–9,000 rpm for 10 minutes.

The DNA pellet was washed with 1 mL of 70% ethanol and centrifuged again at 7,500 rpm for 5 minutes. After discarding the supernatant, the pellet was air-dried and then dissolved in 100 µL of sterile DEPC-treated water. Quantification of the extracted ctDNA was performed using a nanodrop spectrophotometer.

Results and Discussion

The study revealed no statistically significant differences in ctDNA yield between the two anticoagulants tested **(Figure 1)**. The mean ctDNA concentration obtained using *G. sylvestre* was 1022 ng/µL (SD = 35.59 ng/µL), which was comparable to the yield from EDTA-treated samples, measuring 936.33 ng/µL (SD = 128.47 ng/µL). Similarly, both anticoagulants demonstrated comparable results in terms of RNA purity and concentration **(Figure 2)**, indicating that *G. sylvestre* is as effective as EDTA in preserving nucleic acids in blood samples from OSCC patients.

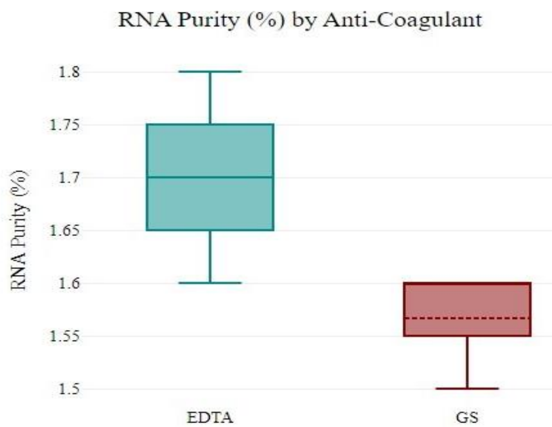

Figure 1. Comparison of RNA purity between *G. sylvestre* and EDTA in OSCC patient blood samples.

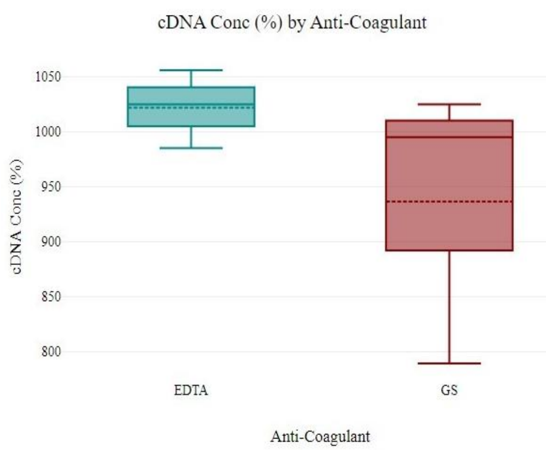

Figure 2. Comparison of ctDNA concentration between *G. sylvestre* and EDTA in OSCC patient blood samples.

G. sylvestre, a plant known for its diverse pharmacological properties, demonstrated anticoagulant activity sufficient to maintain ctDNA integrity during storage and processing. This suggests the potential utility of *G. sylvestre* as a natural alternative to synthetic anticoagulants such as EDTA in molecular studies. Notably, crude *G. sylvestre* extract was used in this study, implying that further purification of its active constituents may enhance ctDNA yield and anticoagulant effectiveness [10-12].

Previous literature has highlighted the in vivo anticoagulant potential of *Gymnema* species, possibly through prolongation of coagulation parameters like PTT and APTT. In this study, *G. sylvestre* demonstrated concentration-dependent anticoagulant effects comparable to EDTA. For example, anticoagulant activity reached a peak at 500 ppm, where clotting times were similar to those achieved with standard EDTA [11-14].

The anticoagulant properties of botanical extracts are typically attributed to their ability to chelate ions or inhibit coagulation-related enzymes. While *G. sylvestre*'s exact mechanism remains undefined, other plants such as *Jatropha gossypiifolia* and *Melastoma malabathricum* exert their effects through protease inhibition, particularly serine protease inhibitors. Additionally, *Melastoma* extracts were found to contain bioactive fractions (F1, F2, F3) with distinct anticoagulant roles, while compounds like coumarin derivatives have been identified as the active components in *Artemisia dracunculus*.

Despite the known pharmacological profile of *G. sylvestre*, no specific compound responsible for its anticoagulant activity has been isolated to date. Future research should aim to identify and characterize these bioactive constituents, which could pave the way for the development of a safe, plant-based anticoagulant suitable for laboratory and clinical use [12-15].

Conclusion

G. sylvestre demonstrated the ability to prevent blood coagulation without compromising ctDNA yield, making it a promising candidate for use as a natural anticoagulant in molecular research. Further studies are warranted to isolate and optimize the active components responsible for this effect, potentially offering a safer, plant-derived alternative to synthetic agents like EDTA.

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