

Herbal Medicine as a Natural Strategy for Combating Oral Squamous Cell Carcinoma

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Abstract: Oral Squamous Cell Carcinoma (OSCC) remains a major health burden in Karnataka, necessitating innovative, integrative therapeutic strategies. This study employed a multidisciplinary approach combining geospatial mapping, phytochemical screening, and nanotechnology. ArcGIS analysis identified OSCC hotspots across Karnataka, while microbial profiling of saliva samples from patients revealed key pathogenic associations. Ethanolic extracts of *Garcinia gummi-gutta*, *Justicia wynaadensis*, and *Thunbergia fragrans* were prepared and evaluated for antimicrobial, antioxidant, and cytotoxic activities. Among them, *Justicia wynaadensis* extract (JwE) exhibited the highest bioactivity, significantly reducing KB cell viability ($IC_{50} = 86.31 \mu\text{g/mL}$) and inducing apoptosis. High-resolution LC-MS analysis identified bioactive flavonoids and terpenoids as potential anticancer constituents. Using these extracts, TiO_2 nanoparticles (St- TiO_2 NPs) were synthesized and structurally characterized. The nanoparticles demonstrated potent cytotoxic effects against CAL 27 oral cancer cells by inducing reactive oxygen species (ROS) generation, reducing AKT1 expression, and inhibiting cell migration. Anti-angiogenic efficacy was confirmed via the chick chorioallantoic membrane (CAM) assay, and in vivo studies in OSCC-induced hamsters revealed marked tumor regression and histological restoration of buccal tissue. These findings underscore the therapeutic promise of *J. wynaadensis*-derived TiO_2 nanoparticles as a novel, natural, and effective approach for OSCC management, integrating traditional medicine with modern nanomedicine.

Keywords: Oral Squamous Cell Carcinoma (OSCC); St- TiO_2 NPs; *Justicia wynaadensis*; AKT1; Oral Patch

I. INTRODUCTION

Cancer has emerged as a global epidemic, claiming millions of lives each year, with oral cancer quietly asserting itself as a particularly insidious threat. Cancer is characterized by the uncontrolled proliferation of cells, which can occur in almost any tissue or organ [1]. Under normal conditions, cells follow a tightly regulated cycle of growth, division, and replacement of damaged or aging cells. However, when these regulatory pathways are disrupted due to genetic mutations it often affects key cell cycle checkpoints, allowing cells to evade normal growth controls and form tumors abnormal masses of tissue that may infiltrate or spread to distant sites via the bloodstream and lymphatic system [2].

Tumors are classified into two broad categories: benign and malignant. Benign tumors are non-cancerous, slow-growing, and do not spread to other parts of the body. They can be completely removed by surgery with minimal risk to the patient. Malignant tumors, being cancerous, may invade nearby tissues or even metastasize to distant organs, which makes it dangerous and fatal [3]. Not all cancers come with solid tumors; hematologic malignancies like leukaemia and certain types of lymphomas affect the blood, bone marrow, and lymph system but do not form solid masses. Understanding these mechanisms is the key to developing treatment strategies and improving patient outcomes. The science of therapy for cancer continuously evolves and includes novel approaches, such as targeted therapies and immunotherapies that help unleash the immune system to combat cancer [4].

Globally, one of the most formidable health challenges is cancer, killing about 8 million people each year and affecting the mental and psychological states of patients, their family members, and caregivers. According to the World Health



Organization, "Cancer is the major cause of morbidity and mortality around the world, accounting for nearly one in six deaths." [5].

The diagnostic landscape of oral squamous cell carcinoma is rapidly evolving with several technologies described below, aiming to improve early detection [1][2].

Liquid Biopsy: This non-invasive technique holds assessments of levels of circulating tumor cells (CTCs), cell-free DNA (cfDNA), and exosomes within blood or saliva. It means liquid biopsies will be an ideal means for information on the dynamics of tumors and real-time monitoring, without the need for traditional biopsies like inserting needles for analysis of cancer. It is imperative because it allows early detection and improves treatment outcomes.

Light-Based Detection Systems: Two even more advanced technologies involve chemiluminescence and OCT. This latter technology uses light to produce high-resolution images of tissue, thereby clinicians can view abnormalities without performing a biopsy. Both hold great promise but have shortcomings: the most significant being that they can often report false positives [5].

Artificial Intelligence (AI): Advances in AI and machine learning are increasingly applied to the processes of enhancing the diagnostic process of OSCC, hence improving detection and, ultimately, increasing the efficacy of treatment in patients [6].

More recently, studies have discovered that saliva-derived exosomal biomarkers, such as miR-210 and miR-24-3p, might be useful in predicting OSCC with sensitivity rates of 92.31% and accuracy values greater than 0.738, respectively [7]. Another interesting development involves artificial intelligence and its integration into the detection process to improve accuracy. For example, in one of the recent clinical studies, AI algorithms analyzed digitized oral cavity images and provided more than 90% accuracy in distinguishing malignant lesions from benign conditions [6,7].

These are significant advancements regarding the early detection of OSCC, critical in effective management, and improved survival rates. The treatment of oral cancer, especially OSCC, has thus become a subject for integrating traditional modalities with innovative therapeutic strategies.

II. LITERATURE REVIEW

Zenobia & Hajishengallis (2015) review of *P. gingivalis* virulence mechanisms (gingipains, fimbriae) and immune subversion that create a pro-tumour microenvironment; useful background linking oral pathogens to cancer-promoting inflammation.

Ha et al. (2016) showed *P. gingivalis* increases invasiveness of OSCC cells via IL-8-mediated upregulation of MMPs, supporting microbial promotion of EMT and metastasis.

Woo et al. (2017) demonstrated that sustained *P. gingivalis* infection confers chemoresistance and enhances metastatic potential of OSCC cells in vitro and in xenografts, highlighting infection's functional impact on therapy response.

Song et al. (2019) oral administration of *P. gingivalis* promotes paclitaxel resistance in OSCC xenografts, reinforcing the clinical relevance of pathogen-tumor interactions.

Rabel et al. (2020) comprehensive reviews begin emphasizing phytochemicals (curcumin, EGCG, resveratrol, quercetin, berberine) for OSCC chemoprevention and therapy; evidence compiled from cell/animal studies showing apoptosis induction, NF-κB inhibition, MMP/EMT suppression.

Chen et al. (2021) cohort study: **salivary *P. gingivalis*** abundance (and specific fimA genotypes) is higher in OSCC patients and correlates with prognosis, supporting saliva as a diagnostic/prognostic biofluid and linking microbiome to OSCC risk.

Protocatechuic acid & polyphenols (Acquaviva et al., 2021; Punvittayagul et al., 2022; Sharma et al., 2022) focused reviews and experimental work on **protocatechuic acid** and other phenolics show pro-apoptotic, anti-metastatic effects (Ras/Akt/NF-κB, MMP downregulation) across cancer models, marking PCA as a promising phytochemical.

Sobocki et al. (2022) review mapping molecular pathways that connect periodontal pathogens (including *P. gingivalis*) to carcinogenesis (IL-8, MMPs, Notch1 activation, CSC features), helping integrate microbiome and phytochemical intervention rationales.



Zhou et al. (2022) described Ru(II)-modified TiO₂ nanoparticles for hypoxia-adaptive photo-immunotherapy in OSCC models — evidence that **metal-oxide nanoplatforms** (including TiO₂) can potentiate anticancer effects and pair well with phytochemicals.

Mukherjee et al. (2023) systematic/critical review on curcumin and its nano-formulations for oral cancer: nano-delivery improves curcumin bioavailability and therapeutic indices in OSCC models.

Li / EGCG reviews (2024) updated summaries of **EGCG (green tea catechin)** actions: antioxidant/epigenetic modulation, inhibition of invasion/angiogenesis, and potential synergy with other phytochemicals in OSCC prevention/therapy.

Clinical/translational evidence growing cohort and tissue studies confirm higher prevalence of *P. gingivalis* in OSCC tissues and saliva, strengthening the microbiome–OSCC association and the rationale for antimicrobial + phytochemical strategies.

The recent papers highlight (a) advanced nanocarriers (TiO₂ and hybrid platforms) to deliver phytochemicals and induce ROS/photothermal effects in OSCC, and (b) integrative approaches combining microbiome modulation, phytochemical therapy, and nanodelivery as near-term translational directions.

III. RESEARCH METHODOLOGY

The objective of the current study is to assess the prevalence and spatial distribution of oral cancer in Karnataka, India, using data from the Kidwai Memorial Institute of Oncology, Bengaluru, Karnataka, India. Further, the study aims to identify and characterize the bacterial communities in the oral cavities of patients with oral cancer, with samples collected from JSS Dental College and Hospital, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India. The study involves biochemical and molecular techniques, including 16S rRNA gene sequencing and phylogenetic analysis, to identify specific bacterial strains and evaluate their potential role in biofilm formation. These findings intended to enhance our understanding of the connection between oral microbiota and oral cancer, while also highlighting the significant public health challenges posed by oral cancer in the Mysuru region

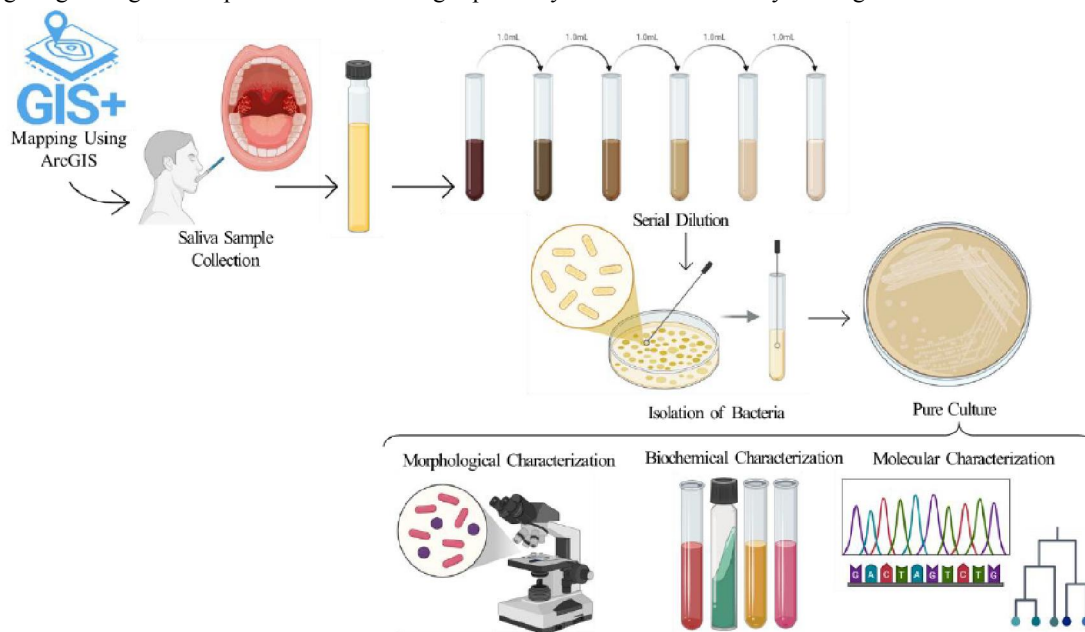


Figure 1: Workflow for Microbial Isolation and Molecular Characterization from Oral Cancer Patient Samples in Karnataka

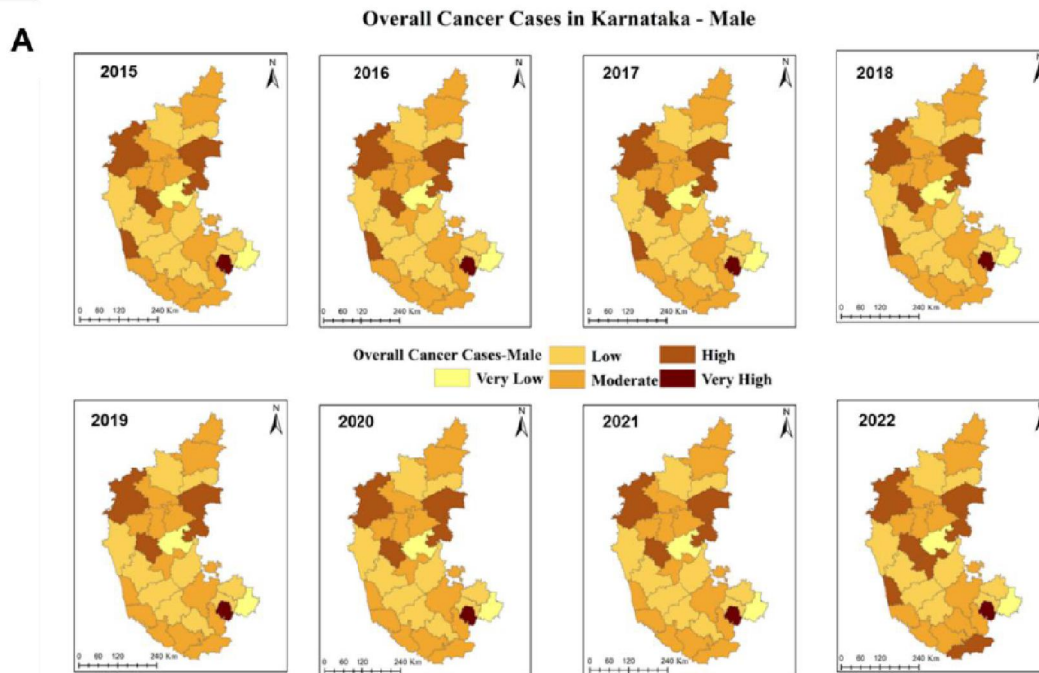
The graphical abstract illustrates the methodology for identifying and analyzing microbial isolates. Initially, GIS mapping was employed to determine the prevalence of oral cancer in Karnataka, followed by the collection of saliva



samples from cancer patients, which were then transferred to a transport medium. The samples underwent serial dilution to reduce microbial load, facilitating easier colony isolation on nutrient agar plates. Microscopic examination allowed for the observation of colony morphology, which was subsequently complemented by biochemical testing to characterize metabolic profiles. Finally, molecular identification was achieved through DNA extraction and sequencing, confirming the identity of each isolate, which was further explored through phylogenetic analysis to elucidate evolutionary relationships among the strains.

IV. RESULTS AND DISCUSSION

The data, procured from Kidwai Memorial Hospital that is from the year 2015-2022 were mapped using ArcGIS software, showing an increase in cancer incidence across Karnataka, with the highest cases reported in Bangalore-Urban for both male and female genders. In this region, the overall cancer cases for males were 6,068 in the year 2015 to 7,932 by 2022, similarly in females the cases increased from 7,445 to 9,865 by the end of the year 2022. Whereas in Kodagu district only a few cases were reported, remaining around 284 for males and showing only a slight increase in females from 395 to 402 between the year 2015 and 2022. Focusing on Mysuru, overall cancer cases in males increased from 1,640 in 2015 to 1,783 in 2022, while cases in females grew from 2,191 to 2,448 as shown in (Figure 2). The spatial analysis highlighted cancer hotspots in urban and densely populated districts, while rural areas showed lower incidences, potentially reflecting differences in healthcare access, diagnostic facilities, or population demographics. Mysore, in particular, displayed a significant cancer burden, underscoring the need for enhanced screening and healthcare services.



B

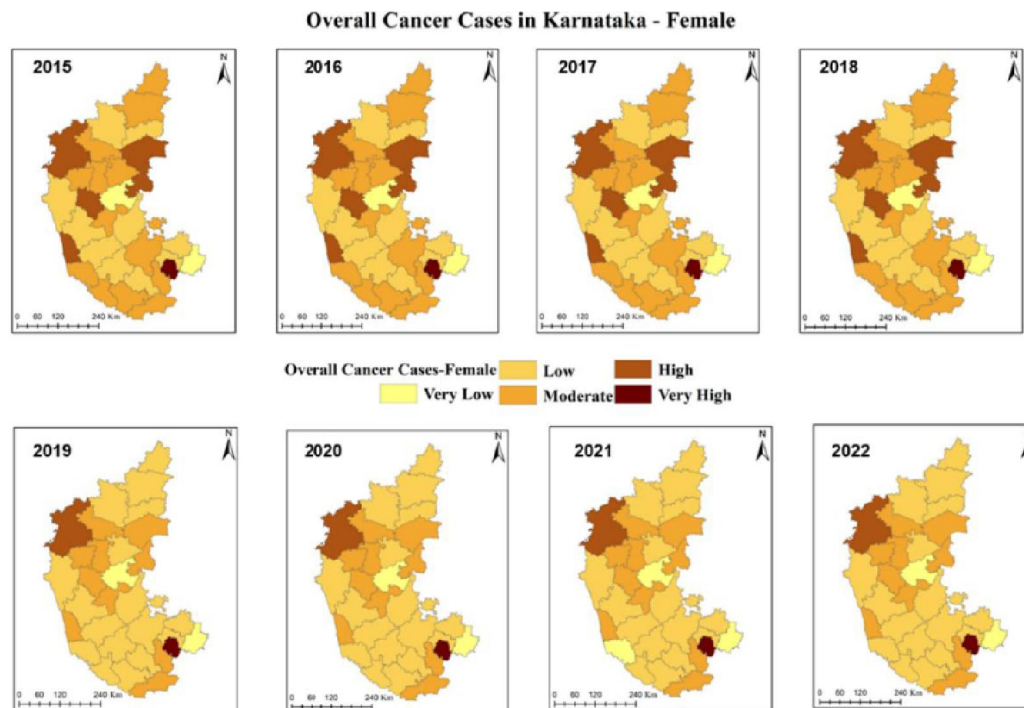


Figure 2. Year-wise Distribution of Cancer Cases Among Males (A) and Females (B) in Karnataka (2015–2022) Based on Incidence Intensity

The map shows over all cancer cases in Karnataka year wise distribution from 2015-22 among males(A), and females (B)in Karnataka, with colour intensity representing incidence: Very Low represented by Light Yellow, Low by Light Orange, Moderate, such as Mysore by Darker Orange, High by Dark Brown, and Very High by Dark Red. This gradation aids in understanding regional disparities in cancer incidence, highlighting areas with varying cancer burdens.

V. CONCLUSION

This study highlights the potential of traditional herbal medicines, particularly *Justicia wynaadensis*, as effective therapeutic agents against OSCC. The promising results from TiO₂NPs as drug delivery vehicles indicate a novel approach to enhancing treatment efficacy. These findings warrant further exploration of herbal extracts and their bioactive constituents in the development of integrated cancer therapies, emphasizing the importance of combining traditional knowledge with modern scientific methods in addressing complex health issues like oral cancer.

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