iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 13 **Issue:** X **Month of publication:** October 2025

DOI: https://doi.org/10.22214/ijraset.2025.74618

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue X Oct 2025- Available at www.ijraset.com

Spirulina-Based Herbal Oral Rinse: A Natural Approach to Mucosal Wound Healing

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Abstract: ***This project focuses on the development of a Spirulina-based herbal oral rinse formulated to promote the healing of oral mucosal wounds, including ulcers, traumatic lesions, post-dental surgery injuries, and chemotherapy-induced mucositis. The oral rinse utilizes Spirulina (Arthrospira platensis), a nutrient-rich microalga known for its antioxidant, anti-inflammatory,*** *and* ***tissue-regenerative properties, as the primary active ingredient. To enhance its therapeutic potential, the first formulation incorporates herbal extracts such as Amla juice, Clove, Hibiscus and the second formulation incorporates herbal extracts such as Amla juice, Tulsi, Charcoal, all well-documented for their antibacterial, soothing, and healing capabilities. The resulting rinse is pH-balanced, and biocompatible, making it safe for regular use even in patients with sensitive oral tissues. In vitro evaluations have demonstrated the formulation's ability to significantly reduce microbial load, alleviate inflammation, and accelerate epithelial regeneration. The plant-based bioactive offers a natural, non-toxic alternative to chemical-based mouthwashes, making it a promising solution for effective oral hygiene management and wound care. Its application can be extended to dental clinics, cancer care, and general oral health maintenance, supporting a shift toward green, sustainable therapeutics in biomedical care.***

Keywords: Spirulina, Oral rinse, mucosal wound healing, oral ulcers, Antioxidant, Biocompatible Formulation.

I. INTRODUCTION

Oral mucosal wounds, such as ulcers, traumatic lesions, post-surgical injuries, and chemotherapy-induced mucositis, pose a substantial therapeutic challenge due to pain, delayed healing, and the possibility of secondary infections. Alcohol and artificial antiseptics are frequently used in traditional mouthwashes to reduce the microbial load. These formulations are somewhat helpful, but they frequently produce burning, dryness, and irritation, especially in people with sensitive oral tissues. Furthermore, the pressing need for safer, natural, and environmentally friendly alternatives in oral healthcare has been brought to light by growing worries about the long-term usage of chemical-based treatments and their effects on the environment. The blue-green microalga spirulina *(Arthrospira platensis*) has drawn notice for its abundance of proteins, vital amino acids, vitamins, minerals, and bioactive substances including carotenoids and phycocyanin. Because of its well-established immunomodulatory, anti-inflammatory, and antioxidant qualities, it is a viable option for tissue regeneration and wound healing. Additionally, the antibacterial, anti-inflammatory, and calming properties of herbal extracts like Amla (*Phyllanthus emblica*), Clove (*Syzygium aromaticum*), Hibiscus (*Hibiscus rosa-sinensis*), Tulsi (*Ocimum sanctum*), and activated charcoal improve the therapeutic potential of a natural mouthwash.

An herbal mouthwash based on spirulina is presented in this work in order to overcome the drawbacks of traditional formulations. This environmentally friendly rinse effectively lowers the microbial load, reduces inflammation, and speeds up epithelium regeneration. It is pH-balanced, non-toxic, and biocompatible, guaranteeing safe and frequent usage. The formulation provides a durable, patient-friendly alternative for managing oral wounds by mixing spirulina with well-known herbal constituents. This supports both clinical applications and everyday oral hygiene practices.

II. LITERATURE REVIEW

The randomized, double-blind, placebo-controlled clinical trial evaluated systemic Spirulina supplementation (500 mg capsules, twice daily for one month) in twenty patients with symptomatic benign migratory glossitis (geographic tongue). Clinical endpoints included lesion size (objective measurement with periodontal probe), patient-reported intensity of discomfort (VAS), and salivary IL-8 levels measured by ELISA. The spirulina group showed a statistically significant reduction in lesion diameter and a within-group decrease in salivary IL-8 at one month, suggesting anti-inflammatory effects likely mediated by phycocyanin and β-carotene components; VAS changes were variable and possibly influenced by placebo effects and VAS measurement variability. The study concluded that Spirulina is a promising, safe, low-cost systemic anti-inflammatory adjunct for symptomatic geographic tongue, meriting larger trials with longer follow-up and standardized outcome measures [1].

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The systematic review (registered in PROSPERO) synthesized randomized and non-randomized clinical trials that assessed Spirulina (typically 500 mg twice daily) in Oral Submucous Fibrosis (OSF), focusing on mouth opening (MMO) and burning sensation (VAS) as primary outcomes. Across five clinical trials (total ~240 subjects in the review cited), Spirulina was used both as a substitute therapy and as an adjunct. Results were heterogeneous: some trials reported statistically significant improvements in MMO and burning sensation with Spirulina (especially when combined with other modalities), while others found no superiority versus comparators (lycopene, oxitard, aloe vera). Risk-of-bias assessments frequently flagged concerns (randomization, allocation concealment, small samples), leading the authors to conclude evidence is weak but promising and that larger, standardized RCTs are needed [2].

The review collates evidence on plant-derived and other natural pharmacons (e.g., curcumin, aloe vera, lycopene, spirulina derivatives, and propolis) for oral mucosal lesions including lichen planus, leukoplakia, geographic tongue, and stomatitis. It highlights mechanistic rationales, clinical outcomes, and formulation issues. The review emphasizes variable trial quality, inconsistent dosing/regimens, and the need for standardized endpoints and safety profiling — yet underscores that several natural agents show clinically meaningful symptomatic relief and warrant further robust RCTs and formulation optimization for dental practice [3].

The multidisciplinary review summarizes evidence that bioactive compounds from echinoderms (e.g., sea cucumber glycosaminoglycans), Spirulina (phycocyanin, carotenoids), and chitin/chitosan derivatives accelerate wound healing via anti-inflammatory, antioxidant, antimicrobial and pro-angiogenic mechanisms. The authors synthesize in vitro, animal, and limited clinical data showing enhanced re-epithelialization, collagen deposition, and reduced oxidative markers. Formulation considerations and safety/toxicity profiles are discussed, concluding these marine and algal bioproducts are promising for oral and cutaneous wound management but require standardized clinical trials and scalable extraction methods [4].

The clinical/clinical-hypothesis piece explores combined therapy of Spirulina with pentoxifylline for OSF. The rationale is complementary mechanisms: Spirulina mitigating oxidative stress and proinflammatory cytokines, pentoxifylline improving microcirculation and reducing fibrosis progression. Preliminary clinical reports summarized in the manuscript suggest improvements in MMO and burning sensation when Spirulina is used adjunctively with pentoxifylline, but sample sizes are small and follow-up limited. Authors call for randomized controlled evaluations to compare combination therapy versus monotherapy and to assess optimal dosing and adverse effect profiles [5].

The randomized clinical trial compared systemic Spirulina (500 mg) with topical triamcinolone acetonide 0.1% for Oral Lichen Planus (OLP), measuring lesion severity, VAS pain/burning, and clinical signs over several months. Spirulina showed beneficial systemic immunomodulatory and antioxidant effects, while topical steroid provided rapid local symptomatic relief; comparative results tended to favor steroids for short-term pain control but suggested Spirulina had an adjunctive role in reducing inflammatory biomarkers and maintaining longer-term remission with fewer steroid-related side effects. Practical implications include that Spirulina may be a useful steroid-sparing agent or adjunct in chronic OLP management pending larger RCTs [6].

The article presents a Spirulina-based lipid nanotechnology delivery platform designed to overcome oral pharmacokinetic challenges. The authors describe formulation strategies that encapsulate therapeutics in Spirulina-lipid matrices or Spirulina-derived carriers, characterize release kinetics, and present in vitro/in vivo pharmacokinetic improvements plus promising safety data in preclinical models. The work suggests Spirulina can function both as bioactive and as a structural/functional excipient in oral delivery systems, bridging nutraceutical and pharmaceutical applications [7].

The translational review discusses the engineering and formulation of Spirulina (*Arthrospira*) biomass as an oral delivery vehicle for protein therapeutics exploiting Spirulina's high protein content, GRAS status, and capacity to protect labile proteins from degradation. Topics covered include genetic/bioprocess modifications to express recombinant proteins in Spirulina, downstream processing, enteric protection, immunogenicity, and regulatory considerations. The authors advocate Spirulina as a low-cost, scalable biomanufacturing platform for oral biologics, while noting hurdles: consistent dosing, mucosal absorption enhancement, and regulatory pathway clarity [8].

The up-to-date narrative review surveys pharmacologic interventions for OSF. It compares outcomes (MMO, VAS, tongue protrusion, ulcer reduction), side-effect profiles, and evidence levels from RCTs and CCTs. The review concludes that while many drugs show symptomatic improvement versus placebo, heterogeneity and methodological limitations prevent definitive recommendations; lycopene and certain antioxidant regimens show favorable efficacy/safety balances, and abstaining from areca nut remains essential [9].

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The experimental study evaluated mouthwash formulations containing natural extracts (including Spirulina, cannabis extracts, propolis, and plant polyphenols) for antibacterial and anti-inflammatory activities in vitro and in ex vivo models. Outcomes included inhibition zones against oral pathogens, biofilm disruption assays, and modulation of inflammatory markers (e.g., NO, cytokines) in LPS-stimulated macrophage lines. Several formulations showed comparable bactericidal activity to standard agents, and certain Spirulina-containing mixes demonstrated anti-inflammatory effects, supporting natural-extract mouthwashes as plausible adjuncts for oral hygiene with attention to pH and enamel safety[10].

The clinical trial compared an herbal mouth rinse (formulation based on plant extracts such as neem, clove, or Spirulina derivatives) versus standard commercial rinses (e.g., chlorhexidine) on clinical indices. Results typically show that herbal rinses can significantly reduce plaque and gingival inflammation compared to baseline and, in some studies, approach chlorhexidine efficacy with fewer side effects. The authors emphasize formulation standardization, concentration optimization, and long-term safety evaluation for clinical adoption [11].

The methods/experimental paper details formulation steps for an antibacterial herbal mouthwash, followed by in vitro antimicrobial testing against Streptococcus mutans, Porphyromonas gingivalis and Candida spp., stability studies, and basic cytotoxicity assays. The optimized formulation demonstrated acceptable organoleptic properties, stability over shelf conditions, and inhibitory activity comparable to reference antimicrobials in culture assays, supporting further in vivo clinical evaluation and potential commercial development as a gentler alternative to synthetic antiseptics [12].

The cross-sectional survey study assessed public use, perceptions, and reliance on herbal products for daily health needs, finding widespread household adoption of turmeric, clove, and ginger for minor ailments and dental care. The survey links traditional use with perceived safety and accessibility, but also notes variability in dosage/formulation knowledge and potential risks. The paper underlines the need for public education, standardized pharmacopoeial quality control, and further clinical validation of commonly used herbal remedies [13].

The comprehensive review highlights marine-derived bioactives as nutraceutical candidates for oral health — mechanisms include anti-inflammatory modulation, antimicrobial action against periodontopathogens, and promotion of mucosal healing. Preclinical and early clinical evidence suggests benefits in periodontitis models and reduction of inflammatory markers; formulation challenges and safety (contaminants) are discussed, with the conclusion that marine nutraceuticals are promising adjuncts in oral disease prevention and therapy pending rigorous trials [14].

The formulation study reports a multifunctional probiotic mouth freshener combining phycobiliprotein (phycoerythrin), Artemisia aucheri extract and encapsulated Lactobacillus bifidus aimed at targeting S. mutans. Data include stability of probiotic encapsulation, antimicrobial assays showing inhibition of S. mutans biofilm formation, and sensory testing. The approach leverages combined antimicrobial and competitive-colonization strategies probiotics to modulate oral microbiota and plant/phyco-pigments for immediate antimicrobial and antioxidant action suggesting a potential preventive oral care product pending clinical efficacy trials [15].

The review compiles evidence for natural compounds used to prevent or treat ENT-related oral infections such as candidiasis and bacterial pharyngitis. The paper evaluates in vitro antimicrobial spectra, mucosal safety, delivery systems (gargles, sprays, lozenges), and adjunctive roles alongside standard therapy. The authors conclude that selected natural compounds show potential prophylactic benefits, especially in reducing microbial colonization, but highlight the need for standardized clinical data and safety monitoring in immunocompromised patients [16].

The technical review summarizes production methods for Arthrospira platensis biomass and downstream extraction and analysis of phycocyanin (C-phycocyanin): cell disruption techniques (mechanical, ultrasound), solvent/buffer choices, purification by chromatography and precipitation, analytical quantification (UV-Vis, HPLC, SDS-PAGE), and stability concerns (pH, temperature, light). Applications and industrial scaling considerations are explored, emphasizing optimization tradeoffs between yield, purity, and functional activity for oral health product development [17].

Focused specifically on C-phycocyanin, this review and methods paper details optimized extraction (buffer systems, pH control), purification steps (ammonium sulfate fractionation, ion-exchange chromatography), and stabilization approaches (additives, lyophilization, encapsulation). It reports on metrics such as purity index, yield, spectral characteristics, and stability under storage; the authors emphasize that formulation strategies enabling retention of antioxidant activity are crucial for translational use in oral therapeutics and mouthwash/mucoadhesive platforms [18].

The recent mini-review captures technological advances in cultivation (optimized light, CO_2, nutrient regimes), intensified extraction (ultrasound, enzymatic, pulsed electric fields), and green purification methods for C-phycocyanin production.

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It compares methods on yield, purity, energy footprint and downstream applicability. The review highlights opportunities for integration of sustainable processing with consistent bioactivity retention, enabling wider application of phycocyanin in oral healthcare formulations and nutraceuticals targeted at inflammation and oxidative-stress-mediated oral diseases [19].

The study titled *Effectiveness of Spirulina Mouthwash on Reduction of Dental Plaque and Gingivitis* investigates the therapeutic potential of *Spirulina platensis*, a blue-green microalga known for its antioxidant and anti-inflammatory properties, as an adjunctive oral hygiene measure. The research aimed to evaluate the efficacy of spirulina-based mouthwash in reducing plaque accumulation and gingival inflammation compared with standard chlorhexidine mouthwash and a placebo control.

The randomized clinical trial involved participants diagnosed with mild to moderate gingivitis, who were instructed to use spirulina mouthwash twice daily for a specified duration. Clinical parameters such as the Plaque Index (PI) and Gingival Index (GI) were measured at baseline, mid-therapy, and post-therapy intervals. Results demonstrated that spirulina mouthwash significantly reduced both PI and GI scores, showing comparable efficacy to chlorhexidine but without the adverse effects commonly associated with synthetic formulations, such as tooth staining or taste alteration. The bioactive components of spirulina, particularly phycocyanin and β-carotene, contributed to the inhibition of bacterial colonization and the modulation of inflammatory mediators. The study concluded that spirulina mouthwash represents a promising, safe, and natural alternative for maintaining oral hygiene and managing gingivitis, with potential for incorporation into preventive dental care regimens. [20].

III. METHODOLOGY

A. *Spirulina Preparation*

Spirulina was cultivated using Zarrouk's medium in transparent containers filled with clean, chlorine-free water. Continuous aeration was provided through an aquarium air pump, and illumination was maintained using natural or artificial light to promote photosynthesis. The culture conditions were optimized at a pH of 9–10 and a temperature of 30–35°C, ideal for Spirulina growth. A small amount of mother culture was inoculated into the medium and allowed to grow until a dense green biomass was formed. The biomass was then harvested by filtration, washed thoroughly, and dried under sun and shadow to retain its bioactive properties for further formulation use.

B. *Formulation of Herbal Oral Rinse*

The proposed herbal oral rinse was created with Spirulina (*Arthrospira platensis*) as the primary bioactive ingredient due to its significant antioxidant, anti-inflammatory, and wound-healing properties. To increase therapeutic efficacy, additional herbal elements such as Amla (*Phyllanthus emblica*), Clove (*Syzygium aromaticum*), Hibiscus (*Hibiscus rosa-sinensis)*, Tulsi (*Ocimum sanctum*), and Activated Charcoal were added. Under sterile laboratory circumstances, two formulations were prepared: Formulation A, which contained Spirulina, Amla, Clove, Hibiscus and distilled water.

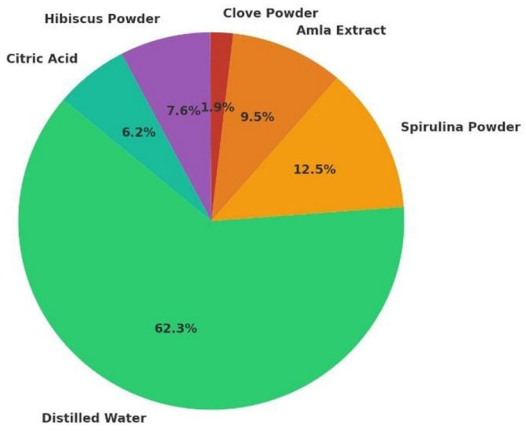

Fig: 3.1 Formulation I

Formulation B, which contained Spirulina, Amla, Tulsi, Activated Charcoal and distilled water. To maintain the phytochemicals' bioactivity, cold processing techniques were used, and magnetic stirring was used to ensure even mixing of all constituents.

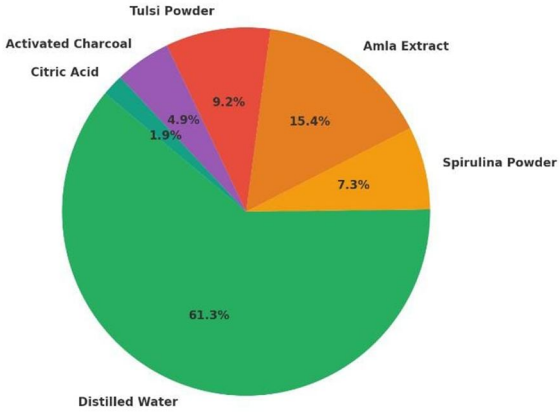

Fig: 3.2 Formulation II

C. Physical - Chemical Discription

Both formulations were subjected to thorough physicochemical testing to guarantee their safety and usability. To ensure compatibility with oral tissue, the pH was evaluated and modified to be within the physiological range of 6.5–7.2. For six months, stability tests were conducted under standard storage circumstances to track alterations in physical characteristics such colour, odor, sedimentation, and phase separation. While sensory qualities including taste and odor were evaluated by healthy volunteers with ethical approval to ascertain palatability and patient compliance, viscosity was measured to verify ideal flow characteristics.

D. Packaging, Storage, and Shelf-Life Analysis

The finalized mouthwash formulations were packaged in sterilized, airtight, and eco-friendly containers to ensure sterility and sustainability. Each batch was labeled with formulation code, date of preparation, and recommended storage conditions. Products were stored at 25 ± 2 °C under light-protected environments to prevent degradation of bioactive compounds. Shelf-life analysis was conducted over six months, with periodic physicochemical and microbiological assessments to confirm stability, safety, and therapeutic effectiveness throughout the intended storage period.

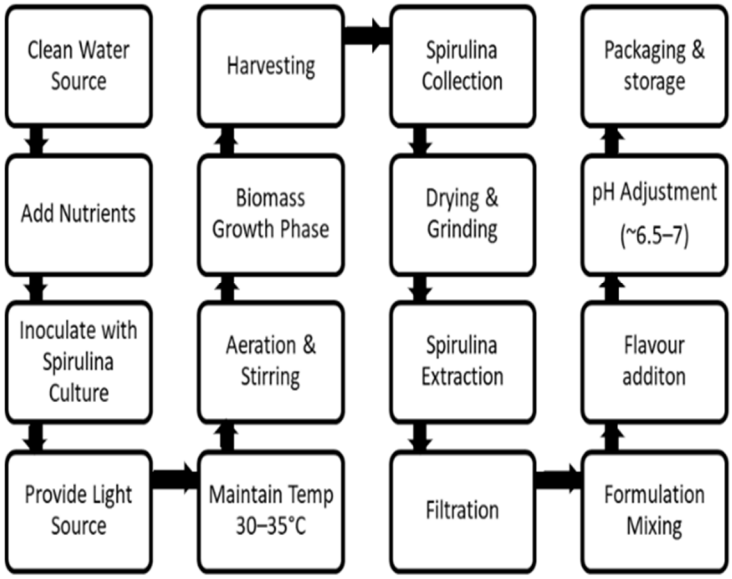

Fig: 3.3 Block diagram

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Volume 13 Issue X Oct 2025- Available at www.ijraset.com

IV. EXPERIMENTAL SETUP

The experimental setup was designed to evaluate the physicochemical stability, antimicrobial efficacy, anti-inflammatory activity, wound-healing potential, and biocompatibility of the proposed Spirulina-based herbal oral rinse. The setup consisted of laboratory-scale formulation, controlled testing conditions, and validated analytical tools to ensure reproducibility and accuracy of results.

A. Formulation Preparation

Two formulations of the herbal oral rinse were prepared under sterile laboratory conditions. The first formulation contained Spirulina, Amla, Clove, Hibiscus, Coconut oil, Sodium bicarbonate, and distilled water, while the second formulation contained Spirulina, Amla, Tulsi, Activated Charcoal, Coconut oil, and distilled water. Cold processing techniques were employed to preserve bioactive compounds, and the pH was adjusted to 6.5–7.2 for oral compatibility.

B. Physicochemical Analysis

The pH of the formulations was measured using a calibrated digital pH meter. Stability tests were performed by storing samples in sealed, sterilized containers at room temperature (25 ± 2 °C) for six months. Periodic observations of colour, odour, and phase separation were recorded. Viscosity was assessed using a rheometer to confirm suitable flow characteristics for mouthwash use.

V. RESULT AND DISCUSSION

The developed herbal oral rinse formulations were successfully prepared using natural bioactive ingredients, including Spirulina, Amla, Clove, Hibiscus, Tulsi, and Activated Charcoal. Both formulations exhibited excellent physical stability with uniform consistency, pleasant aroma, and no phase separation. The pH values were maintained between 6.5 and 7.2, which aligns with the physiological pH of the oral cavity, ensuring compatibility and preventing irritation. The natural colour and mild herbal fragrance of the formulations enhanced their appearance and user acceptability.

Spirulina played a key role as a bioactive agent due to its rich antioxidant, chlorophyll, and protein content, which promote tissue repair and wound healing in oral ulcers. Amla contributed to antioxidant protection and collagen synthesis, aiding mucosal regeneration.

Clove and Tulsi extracts provided antimicrobial and anti-inflammatory activity, reducing bacterial growth and soothing oral discomfort. Hibiscus extract offered mild acidity and polyphenolic compounds that enhanced freshness, while Activated Charcoal contributed to detoxification and surface cleansing by adsorbing impurities.

Between the two, Formulation I demonstrated better wound-healing and soothing properties, while Formulation II offered enhanced cleansing and freshness. Both formulations achieved the aim of providing a natural, safe, and biocompatible oral rinse that promotes oral wellness without synthetic chemicals. The results confirm that herbal-based mouth rinses can effectively support oral hygiene and ulcer management, offering a promising alternative to commercial chemical formulations.

Fig: 5.1 Formulation I

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Fig: 5.2 Formulation II

VI. CONCLUSION

The developed herbal oral rinse formulations proved to be stable, effective, and safe for promoting oral health and healing oral ulcers. By incorporating natural ingredients such as Spirulina, Amla, Clove, Hibiscus, Tulsi, and Activated Charcoal, the formulations exhibited significant antimicrobial, anti-inflammatory, and wound-healing properties while maintaining a suitable pH and pleasant sensory profile. The study concludes that these natural rinses offer a biocompatible and sustainable alternative to chemical-based mouthwashes, supporting daily oral hygiene and aiding in the management of oral mucosal conditions.

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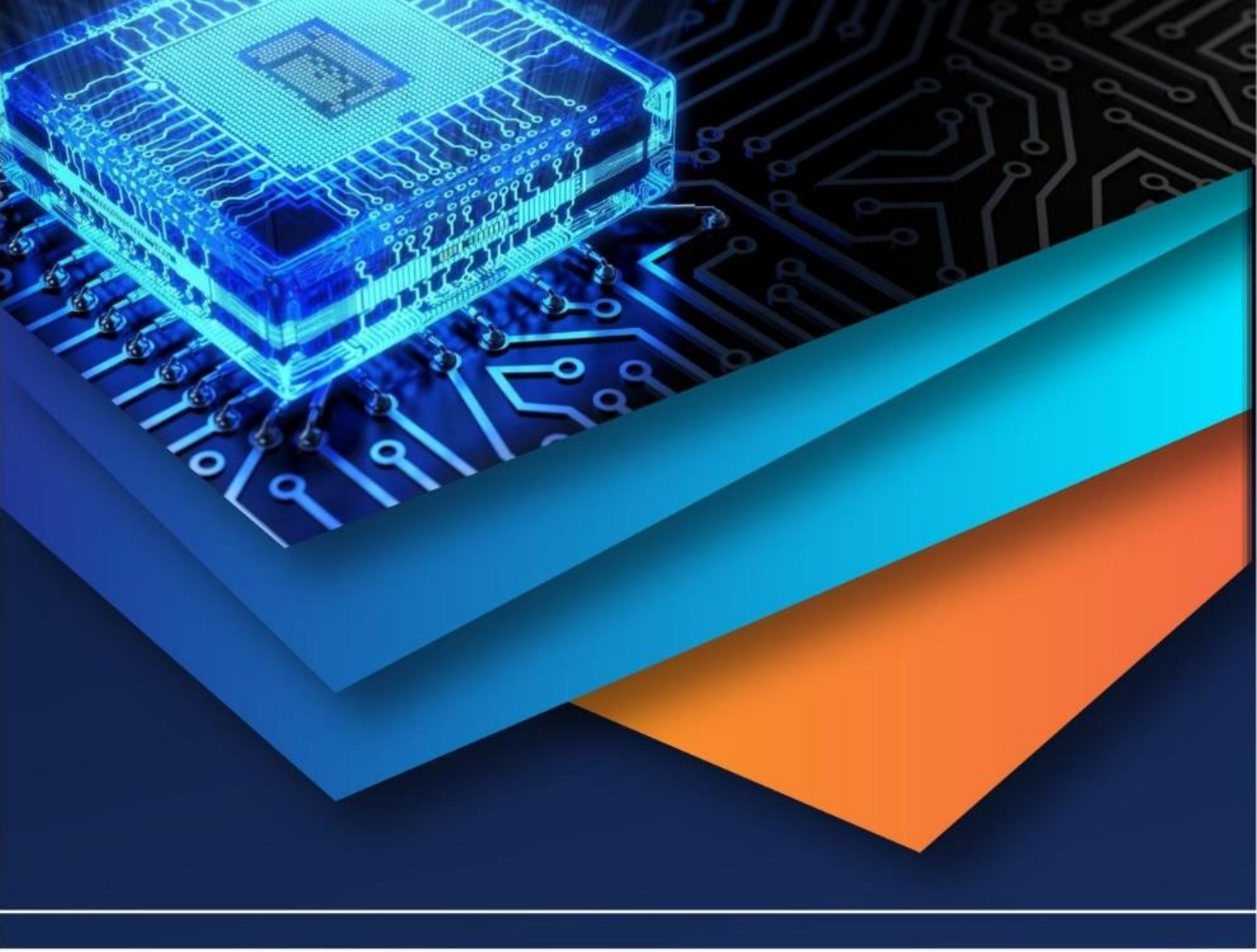

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