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RESEARCH ARTICLE

Exploring the Therapeutic Potential: *In Vitro* Assessment of a Novel Gel with *Withania somnifera* and *Eugenia jambolana* Extracts

Varun Batra and Sankari Malaiappan*

Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha University, Chennai - 600077, Tamil Nadu, India; sankari@saveetha.com

Abstract

Background: *Withania somnifera* (*Ashwagandha*) and *Eugenia jambolana* (Jamun) are traditional medicinal plants with potent antimicrobial, antioxidant, and anti-inflammatory activities. Their combination in a single gel formulation provides a natural and synergistic approach for managing oral infections and inflammatory conditions such as periodontitis. **Aim:** To formulate and evaluate a novel gel containing *W. somnifera* and *E. jambolana* extracts for its antimicrobial, anti-inflammatory, antioxidant, and cytotoxic potential. **Method:** The gel was prepared using a carboxymethyl cellulose-carbopol base incorporating *W. somnifera* and *E. jambolana*-mediated graphene nanoparticles. The formulation was assessed for antimicrobial activity against *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Lactobacillus* sp., and *Candida albicans* by the agar well diffusion method. Anti-inflammatory efficacy was evaluated through bovine serum albumin and egg albumin denaturation assays. Antioxidant potential was examined using DPPH and hydrogen peroxide scavenging assays, while cytotoxicity was determined via the brine shrimp lethality bioassay. **Results:** The formulated gel exhibited significant, concentration-dependent antimicrobial effects, with maximum inhibition zones observed against *S. mutans* and *E. faecalis* (12 mm at 100 µg/mL). Anti-inflammatory assays showed 76% protein denaturation inhibition at 50 µg/mL, comparable to the standard. The gel demonstrated strong antioxidant potential with 86.93% DPPH and 83.4% H_2O_2 radical scavenging at 50 µg/mL. Cytotoxicity evaluation confirmed biocompatibility up to 80 µg/mL. **Conclusion:** The *W. somnifera* and *E. jambolana* gel displayed potent antimicrobial, antioxidant, and anti-inflammatory properties with minimal cytotoxicity, suggesting its potential as a natural, biocompatible adjunct in periodontal therapy.

Major Finding: The formulated gel exhibited broad-spectrum antimicrobial activity, strong antioxidant potential, and biocompatibility, highlighting its promise as a safe plant-based periodontal therapeutic.

Keywords: Antioxidant, Antimicrobial, Anti-inflammatory, *E. jambolana*, Periodontal Therapy, *W. somnifera*

1. Introduction

Periodontal diseases are among the most prevalent oral conditions worldwide and represent a major public health concern. They are primarily infectious in nature and caused by specific microbial complexes inhabiting subgingival biofilms. According to the World Health Organization, nearly 19% of adults globally suffer from severe periodontitis[1]. Gram-negative anaerobic pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* are key etiological agents associated with the initiation and progression of periodontal destruction[2,3]. Persistent infection and dysbiosis of the oral microbiome further exacerbate inflammatory responses, resulting in attachment loss and alveolar bone resorption[4].

Mechanical debridement techniques such as scaling and root planning remain the cornerstone of periodontal therapy. However, these procedures may not completely eliminate pathogenic microorganisms from deeper

*Author for correspondence

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periodontal pockets or inaccessible areas[5]. Adjunctive use of antimicrobial agents has been recommended to enhance clinical outcomes, but increasing antibiotic resistance and adverse effects limit their long-term applicability[6,7]. Consequently, there is a growing interest in herbal and plant-based therapeutics that offer antimicrobial, antioxidant, and anti-inflammatory benefits with improved biocompatibility and minimal side effects[8,9].

Traditional herbal medicine constitutes the primary healthcare modality for nearly 75-80 % of the global population, particularly in developing regions[10]. The World Health Organization (WHO) supports the integration of phytotherapeutic agents into national health systems due to their ecological safety, cultural acceptance, and pharmacological effectiveness[11]. *W. somnifera* (*Ashwagandha*) and *E. jambolana* (Jamun) are well-recognized medicinal plants widely used in Indian traditional medicine for their therapeutic versatility[12,13].

Withania somnifera, belonging to the *Solanaceae* family, contains bioactive withanolides known for their potent anti-inflammatory, immunomodulatory, and antioxidant properties[14,15]. Its use in *Ayurveda* dates back thousands of years as a *Rasayana* (rejuvenator) for enhancing vitality and resistance to stress[16]. Similarly, *E. jambolana* (syn. *Syzygium cumini*), a member of the *Myrtaceae* family, exhibits antibacterial, antioxidant, and antidiabetic activities attributed to its flavonoid-rich seed and leaf extracts[17,18]. These phytoconstituents have demonstrated inhibitory effects against several oral pathogens including *P. gingivalis*, *F. nucleatum*, and *A. actinomycetemcomitans*[19].

Given the synergistic potential of these two medicinal extracts, the present research aimed to formulate and evaluate a novel gel incorporating *W. somnifera* and *E. jambolana* extracts. The study investigates *in-vitro* antimicrobial, anti-inflammatory, antioxidant, and cytotoxic properties, exploring their possible application as a natural adjunct for periodontal therapy.

2. Materials and Methods

2.1 Materials

Withania somnifera (*Ashwagandha*) and *E. jambolana* (Jamun) powders were procured from a certified *Ayurvedic* supplier (India). Carboxymethyl cellulose (CMC), Carbopol 940, and other analytical-grade chemicals were obtained from SD Fine Chemicals Pvt. Ltd., Mumbai. All solutions were prepared using distilled water.

2.2 Preparation of Nanoparticles

An equal quantity (2.5 g each) of *W. somnifera* and *E. jambolana* powders were mixed with 100 mL of distilled water and boiled using a heating mantle. The mixture was filtered through muslin cloth and condensed by gentle heating until the volume was reduced to 2 mL. The concentrated extract was then placed in a microwave oven to induce carbonization and graphene nanoparticle formation. The obtained grapheme powder (0.1 g) was redispersed in 3 mL of distilled water to form the nanoparticle suspension.

Formation of *W. somnifera* and *E. jambolana*-mediated graphene nanoparticles was preliminarily indicated by a visible colour change of the reaction mixture from brown to deep black following microwave-assisted reduction, which is commonly observed during phytochemical carbonization. No further physicochemical characterization (UV-Vis, SEM, FTIR, XRD, DLS) was performed owing to limited instrumentation; detailed morphological and structural analyses will be performed in subsequent studies.

2.3 Preparation of Herbal Gel

Carboxymethyl cellulose (2.5%) was dispersed in 5 mL of distilled water, and Carbopol 940 (2.5%) was separately dissolved in 5 mL of distilled water. Both polymeric solutions were homogenized to achieve uniform viscosity. Subsequently, 1% of the *W. somnifera* and *E. jambolana*-mediated graphene nanoparticle suspension was incorporated into the gel base under continuous stirring until a uniform, smooth formulation was obtained.

The concentration of 1% was selected based on preliminary pilot trials that indicated optimal consistency, homogeneity, and physicochemical stability of the gel without phase separation, while ensuring adequate bioactive concentration for antimicrobial, anti-inflammatory, and antioxidant activity assessments. The prepared gel was stored at 4° C in sterile containers until further evaluation.

2.4 Evaluation of Pharmacological Activities

2.4.1 Anti-inflammatory Activity

The anti-inflammatory potential was determined using the Bovine Serum Albumin (BSA) and egg albumin denaturation assays.

1. For the BSA assay, 0.45 mL of 1% BSA was mixed with 0.05 mL of gel sample (10-50 µg/mL). The pH was

adjusted to 6.3, incubated at 55° C for 30 min, and absorbance was recorded at 660 nm.

2. For the Egg Albumin assay, 0.2 mL of fresh egg albumin and 2.8 mL of phosphate buffer were combined with the test sample (10-50 µg/mL) and incubated similarly. Diclofenac sodium served as the standard reference.

The percentage inhibition of protein denaturation was calculated using the formula:

$$\% \ inhibition = \frac{A_c - A_s}{A_c} \times 100$$

Where, = Absorbance of control and = Absorbance of sample.

2.4.2 Antimicrobial Activity

Antimicrobial efficacy was tested by the agar well diffusion method using *S. aureus*, *S. mutans*, *E. faecalis*, *Lactobacillus* sp., and *C. albicans*. Mueller-Hinton agar plates were inoculated with bacterial suspensions, and 9 mm wells were filled with 25, 50, and 100 µg/mL of gel formulation. Amoxyrite and Fluconazole served as bacterial and fungal standards, respectively. Plates were incubated at 37° C for 24-48 h, and inhibition zones were measured in millimetres.

2.4.3 Antioxidant Activity

Two assays-DPPH and Hydrogen Peroxide (H_2O_2) scavenging-were used to assess antioxidant potential.

1. For the DPPH assay, 200 µL of 0.1 mM DPPH in methanol was mixed with varying gel concentrations (10-50 µg/mL) and incubated for 30 min in the dark. Absorbance was read at 517 nm.
2. For the H_2O_2 assay, the reaction mixture contained 100 µL of 2-deoxy-2-ribose, 1 mL phosphate buffer, and varying concentrations (10-50 µg/mL) of sample. After 1 h at 37° C, absorbance was recorded at 532 nm. Vitamin E was used as the reference antioxidant.

$$\% \ scavenging \ activity = \frac{A_c - A_s}{A_c} \times 100$$

2.4.4 Cytotoxicity Assay

Cytotoxic potential was determined using the Brine Shrimp Lethality Assay. Artificial seawater was prepared by dissolving 2 g of non-iodized salt in 200 mL of water. Ten nauplii were transferred to each well containing 20-100 µL of the gel formulation. Plates were incubated for 24 h under illumination, and the number of live nauplii was counted. The percentage mortality was calculated as:

$$\% \ mortality = \frac{Dead \ nauplii}{Total \ nauplii} \times 100$$

2.5 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± Standard Deviation (SD). Statistical analysis was conducted using one-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant.

2.6 Ethical Statement

The study involved *in vitro* experimentation only; therefore, no animal or human ethical approval was required.

3. Results and Discussion

3.1 Antimicrobial Assessment

According to antimicrobial studies, the novel gel inhibits the growth of a number of bacteria, including *C. albicans*, *S. mutans*, *Lactobacillus* sp., *S. aureus*, and *E. faecalis*. What the study found was that the antibiotic activity depended on the concentration. This means that the zones of inhibition were largest against *S. mutans* and *E. faecalis* at about 12 mm when the concentration was 100 µg/mL (Figure 1a and 1b). *C. albicans* and *Lactobacillus* sp. are especially less likely to grow (<10 mm). All things considered, the gel seems to have broad-spectrum antimicrobial properties, which means it should be able to fight infections caused by both bacteria and fungus.

3.2 Anti-inflammatory Activity

3.2.1 BSA Assay

Both the *W. somnifera* and *S. cumini* (GNPs) sample and the standard exhibit a dose-dependent increase in inhibition, as shown in the BSA assay. At 10 µg/mL, the sample achieves 41% inhibition, which rises to 76% at 50 µg/mL (Table 1). The standard demonstrates slightly higher inhibition, starting at 47% at 10 µg/mL and reaching 84% at 50 µg/mL. This indicates that while both the sample and standard are effective in inhibiting the target, the standard consistently outperforms the sample at each concentration level.



Figure 1. **(a).** Zone of inhibition of the gel formulation against different bacterial strains demonstrating antimicrobial activity.

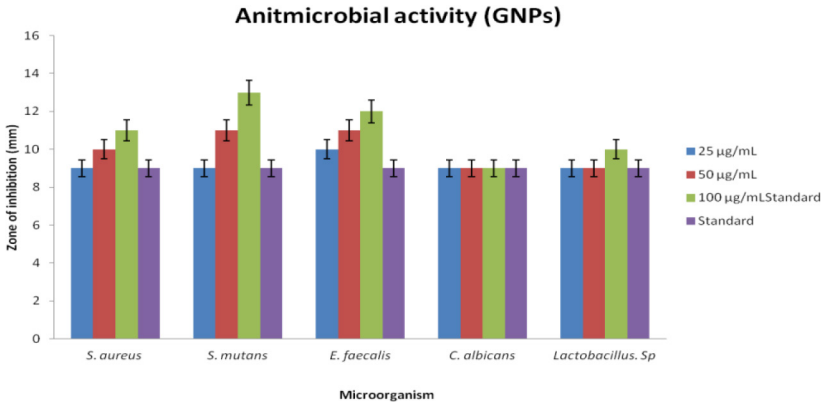


Figure 1. **(b).** Graphical representation of the antimicrobial activity, showing the zone of inhibition at different concentrations.

3.2.2 EA Assay

The (Table 2) shows the inhibition percentages for the *W. somnifera* and *S. cumini* GNPs sample and the standard across different concentrations, similar to the BSA assay. At 10 µg/mL, the sample exhibits 49% inhibition, while the standard shows 55%. As the concentration increases, the inhibition also rises, with the sample reaching 73% and the standard 81% at 50 µg/mL. This indicates a concentration-dependent increase in inhibition, with the standard consistently displaying slightly higher inhibition than the sample at each concentration.

3.3 Antioxidant Activity

3.3.1 DPPH Assay

The (Table 3) shows the antioxidant activity (percentage inhibition) of *W. somnifera* and *S. cumini* GNPs and the standard at various concentrations (10, 20, 30, 40, and 50 µg/mL). At 10 µg/mL, the GNPs exhibit 58.34% inhibition, while the standard shows 66.25%. At 50 µg/mL, inhibition increases to 86.93% for the GNPs and 93.15% for the standard. Both the GNPs and the standard demonstrate a concentration-dependent increase in antioxidant activity, with the standard consistently showing slightly higher inhibition.

3.3.2 H_2O_2 Assay

The H_2O_2 assay compares the antioxidant activity of a standard and a formulation of *W. somnifera* and *S. cumini* (*W. somnifera* and *S. cumini*) combined with Gold Nanoparticles (GNPs). Both treatments show a concentration-dependent increase in H_2O_2 inhibition. The standard demonstrates higher inhibition across

all concentrations, with values ranging from 51.1% at 10 µg/mL to 89.9% at 50 µg/mL (Table 4). The *W. somnifera* and *S. cumini* formulation exhibits slightly lower inhibition, from 47.2% to 83.4% over the same range. Despite this, the formulation shows significant antioxidant potential, particularly at higher concentrations, making it a promising candidate for antioxidant applications.

3.4 Cytotoxicity

The cytotoxicity assessment of the *W. somnifera* and *E. jambolana*-based graphene nanoparticle gel revealed no significant lethality up to 80 µg/mL concentration. Nearly 100 % survival was observed up to 40 µg/mL, with a slight reduction (90%) at 80µg/mL. This indicates that the formulation is biocompatible and safe for biological use (Figure 2).

This study examined the antibacterial, anti-inflammatory, antioxidant, and cytotoxic potential of a new gel formulation combining extracts from *W. somnifera* and *E. jambolana*. The outcomes offer encouraging information about the gel's potential use in periodontal therapy, an area in which oxidative stress, inflammation, and microbial infection all play major roles in the development of disease[27]. These results support the traditional medical applications of both plants and highlight the possibility of plant-based medicines as a substitute for conventional medical care[28].

The gel's antimicrobial efficacy was assessed against many major oral pathogens, including *S. aureus, S. mutans, E. faecalis, C. albicans,* and *Lactobacillus* sp. The study found considerable and concentration-dependent reduction of microbial growth, especially at 100 µg/mL, where the biggest zones of inhibition were detected. The gel effectively inhibited *S. mutans* and *E. faecalis*, two prevalent bacteria that cause dental plaque and endodontic infections, with inhibition zones of up to ~12 mm[29].

This shows that the gel may be beneficial at lowering microbial load in periodontal pockets, which is a key goal of periodontal therapy[30]. *E. jambolana* extracts, which are high in flavonoids and recognized for their broad-spectrum antibacterial activity, are likely to have contributed significantly to this outcome[17]. Additionally, the inhibitory effects on *C. albicans* highlight the gel's potential to manage fungal infections, which are becoming increasingly prevalent in immunocompromised individuals[31]. The broad-spectrum antimicrobial efficacy

Table 1. Inhibition of Bovine Serum Albumin (BSA) denaturation by *W. somnifera*- and *E. jambolana*- based graphene-nanoparticle gel compared with diclofenac sodium at different concentrations (µg/mL)

Concentration (µg/mL)	Gel Formulation	Standard
10	41	47
20	53	60
30	65	72
40	72	78
50	76	84

Table 2. Inhibition of egg-albumin denaturation by the herbal gel and diclofenac sodium at different concentrations (µg/mL)

Concentration (µg/mL)	Gel formulation	Standard
10	49	55
20	59	64
30	62	69
40	64	72
50	73	81

Table 3. DPPH radical-scavenging activity (% inhibition) of the gel formulation and standard antioxidant at various concentrations (µg/mL)

DPPH	Standard	*W. somnifera* and *S. cumini* (GNPs)
10	66.25	58.34
20	78.52	71.45
30	85.63	79.62
40	88.68	82.41
50	93.15	86.93

Table 4. Hydrogen-peroxide (H_2O_2) scavenging activity (% inhibition) of the gel formulation and standard antioxidant at different concentrations (µg/mL)

H_2O_2	Standard	*W. somnifera* and *S. cumini* (GNPs)
10	51.1	47.2
20	56.9	51.3
30	66.1	60.4
40	78.8	72.3
50	89.9	83.4

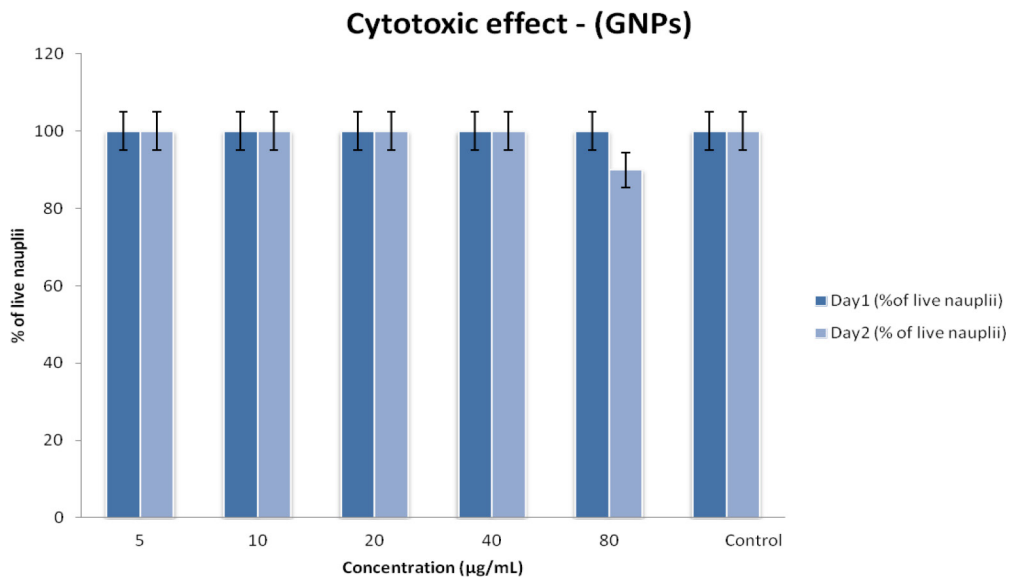


Figure 2. Brine shrimp lethality assay demonstrating the cytotoxicity profile of the gel formulation at different concentrations.

of this gel makes it a potential candidate to reduce reliance on antibiotics, addressing the growing concern over antimicrobial resistance[9].

Both the bovine serum albumin and the egg albumin denaturation assays were used to validate the gel's anti-inflammatory qualities[32]. The anti-inflammatory efficacy of the gel was demonstrated by the dose-dependent reduction of protein denaturation[33]. Diclofenac, a common anti-inflammatory drug, demonstrated 84% inhibition at the same concentration as the gel, which was only 76% inhibited at the maximum tested concentration of 50 µg/mL[34]. Due to the presence of withanolide, *W. somnifera* also referred to as *ashwagandha*, has strong anti-inflammatory qualities[16]. Particularly important in periodontal disease, where inflammation exacerbates tissue destruction, these chemicals modulate the body's inflammatory response and suppress pro-inflammatory cytokines[35]. The gel has the potential to alleviate periodontal tissue damage, minimize gingivitis, and facilitate healing by blocking inflammatory mediators, making it a valuable adjunct in the management of chronic periodontitis[36].

The gel showed significant antioxidant activity in addition to its anti-inflammatory qualities, as shown by the DPPH and H_2O_2 scavenging tests[37]. The gel demonstrated a rise in free radical scavenging that was concentration-dependent, with an 86.93% inhibition in the DPPH assay at 50 µg/mL, which was similar to the standard ascorbic acid value of 93.15%. Reactive Oxygen Species (ROS) enhance inflammatory responses and induce tissue breakdown under oxidative stress, which is recognized as a pathogenesis factor for periodontal disease[38]. Both *E. jambolana* and *W. somnifera* provide antioxidant qualities to the gel that are essential for scavenging free radicals and averting oxidative damage[39]. This antioxidant impact can help regenerate tissue and shield periodontal tissues from further deterioration, particularly in individuals with chronic periodontitis, where oxidative stress is a major factor in the advancement of the illness[40].

The Brine Shrimp Lethality Assay, a dependable and simple technique to gauge the safety of novel formulations, was used to measure the cytotoxicity of the gel[41]. Even at the highest tested dose, the gel showed just a little reduction in nauplii viability and no discernible cytotoxic effects up to 80 µg/mL. This suggests that the gel is safe and biocompatible for possible medical application. Since a formulation for periodontal treatment may be applied over extended periods of time or come into close touch with gingival and oral mucosal tissues, biocompatibility is essential[42]. This formulation's potential for usage in clinical settings is further supported by the fact that it is non-toxic.

Withania somnifera and *E. jambolana* gel presents a viable substitute for traditional periodontal disease therapies because of its antibacterial, anti-inflammatory, and antioxidant characteristics, as demonstrated by this

study[43]. The mainstay of modern periodontal treatments is mechanical debridement (root planning and scaling) in conjunction with chemical agents, including antibiotics or antiseptics. Nevertheless, these therapies have drawbacks, especially in light of the growing antibiotic resistance and worries about long-term security. With none of the drawbacks of long-term antibiotic use, plant-based substitutes like this innovative gel have the dual benefits of treating microbial infections and regulating the host's inflammatory response[44].

However, this study has significant shortcomings that must be addressed. The findings are mostly based on *in vitro* experiments, which do not adequately simulate the complex biological milieu of the mouth cavity. *In vivo* studies are required to assess the gel's efficacy in real-world therapeutic settings, where biofilm formation, salivary enzymes, and patient-specific immunological responses can all have a substantial impact on therapy outcomes. Furthermore, the gel's antimicrobial activity was tested against a specific group of oral pathogens, and its efficacy against a larger variety of bacteria, particularly those involved in polymicrobial diseases, has to be determined.

Furthermore, the study did not assess physicochemical characterization (SEM, FTIR, XRD) and *in vivo* validation. Future research will focus on structural characterisation, biocompatibility on mammalian cells, and animal-model testing for periodontal wound healing. The gel formulation's long-term stability or bioavailability in the oral environment, both of which are crucial to ensuring that the active chemicals remain effective during treatment.

4. Conclusion

The present study successfully formulated and evaluated a novel herbal gel containing *W. somnifera* and *E. jambolana*- mediated graphene nanoparticles. The formulation demonstrated strong antimicrobial, anti-inflammatory, and antioxidant activities, with minimal cytotoxicity in the brine-shrimp assay. These effects may be attributed to the synergistic action of bioactive phytoconstituents and the graphene matrix, which enhances stability, bioavailability, and surface interaction of active compounds.

Collectively, the results indicate that the developed gel possesses significant therapeutic potential as a biocompatible, plant-derived adjunct for periodontal and oral wound-healing applications. Its ability to inhibit microbial growth, neutralize oxidative stress, and prevent protein denaturation suggests usefulness in managing inflammation and promoting tissue repair.

Further *in-vivo* and clinical studies, along with detailed physicochemical characterization (SEM, FTIR, XRD) are recommended to substantiate its efficacy, optimize dosage, and confirm safety before clinical translation.

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6. Author Contribution

Varun Batra: Conceptualization, investigation, data curation, and writing- original draft preparation. Mr. Rajesh: Methodology and formulation of product. Dr. Arvina: Writing-review and editing. Dr. Sankari Malaiappan: Visualization and supervision. All authors have read and agreed to the published version of the manuscript.

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