Available online on 15.10.2026 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Evaluation of the Anti-Inflammatory, Analgesic and Antioxidant Activities of *Achillea millefolium* L. and *Psidium guajava* L. Herbal Gargle Development for Oral Mucositis

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Article Info:



Article History:

Received 24 July 2026
Reviewed 10 Sep 2026
Accepted 29 Sep 2026
Published 15 Oct 2026

Cite this article as:

Gupta P, Kori ML, Evaluation of the Anti-Inflammatory, Analgesic and Antioxidant Activities of *Achillea millefolium* L. and *Psidium guajava* L. Herbal Gargle Development for Oral Mucositis, Journal of Drug Delivery and Therapeutics. 2026; 16(10):72-81 DOI: <https://doi.org/10.22270/jddt.v16i10.8047>

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Abstract

Oral mucositis is a painful inflammatory and ulcerative complication of anticancer therapy. Its pathogenesis involves treatment-induced cellular injury, reactive oxygen species, inflammatory signaling, epithelial disruption, ulceration and delayed healing. The present article integrates the in-vivo pharmacological and formulation findings of a work investigating *Achillea millefolium* L. and *Psidium guajava* L. for oral mucositis management. Ethanolic extracts were evaluated in carrageenan-induced paw edema and hot-plate models, followed by development of five herbal gargle formulations and evaluation in a methotrexate-induced oral mucositis model. Both extracts demonstrated dose- and time-dependent anti-inflammatory and analgesic responses. At 4 h, EAM 200 mg/kg produced 62.21% inhibition of paw edema, while EPG 200 mg/kg produced 65.21%; indomethacin produced 80.11%. In the hot-plate model, EAM 200 mg/kg increased reaction time to 5.20 s at 90 min and EPG 200 mg/kg to 4.98 s, compared with 7.89 s for pentazocine. Among five gargles, F3 demonstrated pH 6.45, viscosity 9.64 cP, minimal sedimentation, excellent redispersibility, low foam formation, favorable stability and the lowest microbial load. In the oral mucositis model, F3 was associated with recovery of body weight, improved food intake, reduction of mucositis score from 3 in disease control to 1, 0% mortality, improved hematological parameters and restoration of tongue architecture on histopathology. The findings support F3 as a promising preclinical herbal gargle candidate while emphasizing the need for standardized and controlled studies.

Keywords: *Achillea millefolium*; *Psidium guajava*; herbal gargle; oral mucositis; carrageenan edema; hot plate; methotrexate; histopathology; antioxidant; anti-inflammatory.

1. Introduction

Oral mucositis (OM) is one of the most clinically important and distressing complications associated with anticancer therapy, particularly chemotherapy, radiotherapy of the head and neck region, and hematopoietic stem-cell transplantation. It is characterized by erythema, epithelial atrophy, inflammation, ulceration and pain of the oral mucosa¹⁻³. The condition can interfere substantially with eating, drinking, swallowing and speaking and, in severe cases, may contribute to infection, hospitalization, nutritional compromise and interruption or modification of anticancer treatment. The clinical importance of OM has stimulated considerable interest in preventive and supportive therapies that can reduce mucosal injury while maintaining patient comfort and quality of life⁴.

The pathogenesis of oral mucositis is complex and involves a sequence of biological events rather than simple epithelial injury. The currently accepted model includes initiation, primary damage response, signal amplification, ulceration and healing. Generation of

reactive oxygen species (ROS) following exposure to cytotoxic therapy is an important early event that contributes to cellular injury and activation of inflammatory signaling pathways. Subsequent release of inflammatory mediators, tissue damage and changes in the oral microbial environment further amplify mucosal injury. Thus, oxidative stress, inflammation, epithelial damage and pain are closely interconnected components of OM. This multifactorial pathogenesis provides a rational basis for investigating agents possessing antioxidant, anti-inflammatory, analgesic and tissue-protective properties⁵⁻⁹.

Despite advances in supportive care, management of oral mucositis remains challenging. Current approaches include meticulous oral hygiene, nutritional support, analgesics, topical preparations, anti-inflammatory agents and selected protective or biological interventions. Evidence-based guidelines have been developed by the Multinational Association of Supportive Care in Cancer and the International Society of Oral Oncology (MASCC/ISOO); however, recommendations differ according to the cancer

treatment modality and clinical setting. Benzydamine mouthwash, for example, has demonstrated usefulness in selected patients receiving radiotherapy, whereas evidence for several other interventions remains insufficient or context-dependent. Consequently, there remains a need for safe, locally acting and potentially multifunctional preparations that can address several pathological components of mucosal injury simultaneously¹⁰⁻¹².

Natural products have attracted increasing attention as potential supportive agents in oral mucositis because medicinal plants contain chemically diverse constituents capable of influencing multiple biological pathways. Phenolic compounds, flavonoids, tannins, terpenoids and other secondary metabolites may neutralize free radicals, modulate inflammatory mediators, stabilize cellular membranes and contribute to antimicrobial or wound-healing effects. A systematic review of medicinal plants used in oncotherapy-induced mucositis reported promising findings for several herbal interventions, including improvements in mucosal lesions and pain symptoms. Similarly, a systematic review of natural agents highlighted the increasing interest in herbal approaches because of their potential therapeutic benefits and comparatively favorable tolerability. These observations support continued investigation of traditionally used medicinal plants, while emphasizing the need for systematic pharmacological evaluation and standardized formulation¹³⁻¹⁵.

Achillea millefolium L., commonly known as yarrow, is a perennial medicinal plant belonging to the family Asteraceae. It has a long history of traditional use for conditions involving inflammation, wounds, gastrointestinal disturbances and pain. Phytochemical investigations have demonstrated the presence of flavonoids, phenolic acids, terpenoids, sesquiterpene lactones and essential-oil constituents. These compounds are considered important contributors to the broad biological activity of the plant. Contemporary pharmacological investigations have reported antioxidant, anti-inflammatory, antimicrobial, antispasmodic and wound-healing properties of *A. millefolium*. Potential anti-inflammatory mechanisms include modulation of cyclooxygenase-related pathways, prostaglandin formation and other inflammatory processes, while its phenolic and flavonoid constituents may contribute to free-radical scavenging activity. A recent comprehensive review further emphasizes the relationship between the phytochemical profile of *A. millefolium* and its antioxidant and anti-inflammatory potential¹⁶.

Psidium guajava L., commonly known as guava, belongs to the family Myrtaceae and is widely distributed in tropical and subtropical regions. Although the fruit is primarily recognized as a food, the leaves have a long history of medicinal use. Guava leaves contain a diverse range of phytoconstituents, including flavonoids, phenolic acids, tannins, terpenoids, alkaloids and other polyphenolic compounds. Quercetin, catechin, rutin, kaempferol, gallic acid, chlorogenic acid and related

compounds have been reported among the bioactive constituents of the leaves. These constituents provide a plausible basis for the antioxidant, anti-inflammatory and antimicrobial activities associated with guava leaves¹⁷.

P. guajava leaves possess substantial pharmacological potential relevant to oral health. Guava leaf extracts have demonstrated antimicrobial activity against several oral microorganisms and have been investigated for potential anti-plaque and periodontal applications. Their traditional use for toothache is also consistent with reported analgesic, anti-inflammatory and antimicrobial properties. In addition, recent evidence indicates that guava-leaf phenolics and flavonoids can contribute to antioxidant and anti-inflammatory effects, while several studies have suggested modulation of inflammatory mediators and pathways such as prostaglandins, cyclooxygenase-2, nitric oxide and inducible nitric oxide synthase¹⁸.

The selection of *A. millefolium* and *P. guajava* for investigation is therefore supported by complementary pharmacological characteristics. The phytoconstituents of both plants provide potential mechanisms for controlling oxidative stress and inflammatory responses, while their reported antimicrobial and analgesic properties may be particularly valuable in maintaining oral mucosal health. Importantly, the use of two medicinal plants may provide a broader pharmacological spectrum than a single botanical ingredient, although such a combination requires systematic experimental validation rather than assumptions of synergism.

Antioxidant activity is particularly relevant because excessive ROS generation contributes to the initiation and amplification phases of mucosal injury. Accordingly, evaluation using complementary free-radical scavenging models such as DPPH, nitric oxide, hydroxyl-radical and superoxide-radical assays can provide useful preliminary evidence of the antioxidant potential of plant extracts. Similarly, anti-inflammatory and analgesic investigations can help determine whether the extracts possess pharmacological effects capable of addressing inflammation and pain, two major clinical manifestations of mucositis¹⁹.

A herbal gargle represents a rational dosage form for local oral application because it permits direct contact of plant-derived constituents with the oral mucosa and can be readily used as part of routine oral care. Unlike systemic administration, a locally applied gargle may provide an opportunity to target the affected oral tissues while minimizing unnecessary systemic exposure. Recent evidence comparing herbal and conventional mouthwashes indicates continuing scientific interest in herbal mouthwash preparations for radiation-induced oral mucositis, although additional well-controlled studies are required to establish efficacy and standardization²⁰.

Therefore, the present investigation was designed to evaluate the anti-inflammatory, analgesic and antioxidant potential of *Achillea millefolium* L. and

Psidium guajava L., with particular emphasis on their possible application in the management of oral mucosal injury. The study further aimed to develop and evaluate a herbal gargle containing the selected plant extracts. The overall approach integrates pharmacognostical and phytochemical characterization with in vitro antioxidant and anti-haemolytic investigations, in vivo anti-inflammatory and analgesic evaluation, and pharmaceutical assessment of the developed gargle. Such an integrated investigation may provide a scientific basis for the traditional use of these plants and support the development of a locally applicable, plant-based supportive preparation for oral mucositis

2. Materials and Methods

Experimental Animals

Wistar albino rats of either sex, weighing approximately 180–220 g, were used for the in-vivo pharmacological investigations. The animals were housed under standard laboratory conditions with controlled temperature, adequate ventilation and a regular light/dark cycle. Animals were provided standard laboratory pellet diet and water ad libitum throughout the experimental period. Before commencement of the experiments, the animals were allowed to acclimatize to the laboratory environment. The animals were randomly allocated into appropriate experimental groups, and efforts were made to minimize animal discomfort and the number of animals used. The experimental procedures were performed during the daytime under standardized environmental conditions.

Preparation of Plant Extracts

Leaves of *Achillea millefolium* L. and *Psidium guajava* L. were collected, authenticated botanically and subjected to appropriate processing before extraction. The dried plant materials were powdered separately and extracted using suitable solvents. The ethanol extracts of *A. millefolium* and *P. guajava* were designated EAM and EPG, respectively. The obtained extracts were concentrated under reduced pressure at a controlled temperature and stored in suitable airtight containers until further investigation.

Acute Oral Toxicity Study

Acute oral toxicity studies were performed to determine the preliminary safety of the ethanol extracts of *A. millefolium* (EAM) and *P. guajava* (EPG). The procedure was conducted according to the relevant OECD acute oral toxicity guideline. The extracts were administered orally at a limit dose of 2000 mg/kg body weight. Following administration, the animals were observed individually and continuously during the initial period and subsequently at regular intervals for general behavioural and toxicological changes

In-Vivo Anti-Inflammatory Activity

Carrageenan-Induced Hind-Paw Edema

The anti-inflammatory activity of EAM and EPG was investigated using the carrageenan-induced hind-paw edema model, a widely used experimental model for evaluating acute peripheral inflammation. The animals

were divided into appropriate treatment groups comprising a normal/control group, disease or carrageenan control group, reference drug group and extract-treated groups. EAM and EPG were administered orally at doses of 100 and 200 mg/kg body weight. Indomethacin at a dose of 10 mg/kg body weight was used as the reference anti-inflammatory drug. Acute inflammation was induced by subplantar administration of carrageenan into the hind paw. The volume of the affected paw was measured immediately before carrageenan administration and subsequently at 1, 2, 3 and 4 h after induction of inflammation. Paw edema was expressed as the change in paw volume relative to the initial measurement.

In-Vivo Analgesic Activity

Hot-Plate Reaction-Time Method

The analgesic activity of EAM and EPG was evaluated using the hot-plate reaction-time method. This model was used to assess the response of the experimental animals to a standardized thermal nociceptive stimulus. The animals were divided into suitable treatment groups. EAM and EPG were administered orally at doses of 100 and 200 mg/kg body weight. Pentazocine at a dose of 100 mg/kg body weight was used as the reference analgesic drug. Reaction times were recorded at baseline and at 30, 60 and 90 min following treatment. An appropriate cut-off period was maintained to prevent tissue injury due to prolonged exposure to the thermal stimulus.

Formulation of Herbal Gargles

Five herbal gargle formulations, designated F1–F5, were prepared using the ethanol extracts of *A. millefolium* and *P. guajava*. The formulations were designed by varying the relative proportions of EAM and EPG while maintaining the total herbal extract concentration at 10 g/100 mL. Glycerin was incorporated as a humectant and to improve mouthfeel, while polysorbate 80 was used as a solubilizing/wetting agent. Sodium benzoate was incorporated as a preservative. Peppermint oil was included as a flavouring and refreshing agent, and purified water was used as the vehicle. For preparation, the required quantities of EAM and EPG were accurately weighed according to the formulation design. The extracts were separately triturated or dispersed with a suitable quantity of the vehicle and solubilizing agent to obtain a uniform dispersion. Glycerin was then incorporated with continuous mixing. Sodium benzoate was dissolved separately in a suitable portion of purified water and added to the formulation. Peppermint oil was incorporated with adequate mixing to ensure uniform distribution. The resulting mixture was transferred to a calibrated volumetric vessel and the volume was made up to 100 mL with purified water. The formulation was mixed thoroughly until a homogeneous herbal gargle was obtained and then filled into suitable, clean and labelled containers. The prepared formulations were stored under appropriate conditions until evaluation.

Evaluation of Herbal Gargle Formulations

The formulations were evaluated for colour, appearance, odour, taste, uniformity, precipitation, phase separation and visible particles. The pH of each formulation was measured using a calibrated digital pH meter. The electrode was rinsed, immersed in the sample and the stabilized pH was recorded. Viscosity was measured using a suitable viscometer under controlled conditions. Results for F1–F5 were recorded and compared. Specific gravity was determined using a pycnometer by comparing the weight of a known volume of formulation with an equal volume of purified water. A known quantity of gargle was evaporated to remove water and volatile matter. The remaining residue was weighed and expressed as percentage total solids. Formulations were allowed to stand and sediment formation was observed. Redispersibility was assessed by gentle shaking, with uniform and rapid redispersion considered desirable. A measured quantity of formulation was shaken under standardized conditions. Foam formation and its persistence were observed. Formulations were stored under suitable conditions and periodically examined for changes in colour, odour, appearance, pH, viscosity, sedimentation and phase separation. The formulations were tested under aseptic conditions using suitable culture media to assess microbial contamination and overall microbiological quality.

Methotrexate-Induced Oral Mucositis Model

Induction of Oral Mucositis

The protective and therapeutic potential of the selected herbal gargle formulation was further investigated using an experimental model of methotrexate-induced oral mucosal injury. Animals were randomly divided into experimental groups comprising a normal control group, disease-control group, standard-treatment group and F3-treated group. Oral mucosal injury was induced using methotrexate according to the established experimental protocol adopted for the study. The disease-control animals received the mucositis-inducing treatment without the herbal intervention, whereas the treatment group received the optimized F3 herbal gargle formulation. The normal control group was maintained without induction of mucosal injury. The standard group received the selected reference treatment used for comparison. Treatment was administered according to the experimental schedule, and the animals were monitored throughout the study for changes in general condition and disease-associated manifestations.

Assessment of Body Weight

Body weight was recorded at baseline and at predetermined intervals throughout the experimental period. Changes in body weight were used as a general indicator of systemic condition and treatment response. The percentage change in body weight, where applicable, was calculated by comparing the body weight recorded at each observation point with the initial body weight. Reduction or failure to maintain body weight in the disease-control group was

interpreted as an indicator of systemic effects associated with mucositis, whereas preservation or recovery of body weight in treated animals was considered a favourable response.

Assessment of Food Intake

Food consumption was monitored throughout the experimental period. The quantity of food provided and the remaining quantity were recorded at predetermined intervals, allowing estimation of daily food intake. Reduced food consumption was considered an indirect indicator of oral discomfort and impaired feeding associated with mucosal injury. Improvement in food intake in the treatment group was therefore considered an additional indicator of beneficial activity of the herbal formulation.

Evaluation of Oral Mucositis Score

The severity of oral mucositis was assessed by macroscopic examination of the oral cavity and tongue using a standardized scoring system. The oral mucosa was examined for erythema, ulceration, inflammation, tissue damage and other visible pathological changes. The severity of mucosal injury was graded according to the predefined experimental scoring criteria. Animals in the normal group were expected to show an intact and healthy oral mucosa, whereas the disease-control group was expected to exhibit prominent mucosal injury. Scores obtained for the standard and F3-treated groups were compared with the disease-control group to determine the extent of protection or recovery. A reduction in mucositis score relative to the disease-control group was considered evidence of therapeutic activity.

Mortality and General Observations

Animals were observed throughout the experimental period for mortality and treatment-related behavioural or physical abnormalities. Particular attention was given to changes in activity, grooming, posture, feeding behaviour, locomotion and other apparent signs of distress. Mortality, when present, was recorded for each experimental group. Animals that died before completion of the experimental period were subjected to appropriate pathological examination wherever applicable, and tissue specimens were collected for histopathological assessment according to the experimental protocol.

Hematological Evaluation

At the end of the experimental period, blood samples were collected from the experimental animals using an appropriate collection procedure. The blood samples were processed for hematological evaluation. The hematological parameters assessed included the relevant blood-cell indices used in the study, such as haemoglobin concentration, red blood cell count, white blood cell count and other applicable hematological parameters. The values obtained from the disease-control group were compared with those of the normal, standard and F3-treated groups to determine the systemic effect of methotrexate-induced mucosal injury

and the potential corrective effect of the herbal treatment.

Histopathological Examination of Tongue

At the end of the experimental period, the animals were humanely sacrificed according to the approved experimental procedure. Tongue tissues were carefully excised and examined macroscopically for visible abnormalities. The collected tongue specimens were immediately immersed in 10% neutral buffered formalin for adequate fixation. After fixation, the tissues were subjected to routine histopathological processing involving dehydration through graded concentrations of alcohol, clearing with a suitable clearing agent and embedding in paraffin wax.

Paraffin-embedded tissue blocks were sectioned using a microtome to obtain thin tissue sections. The sections were mounted on clean glass slides, deparaffinized and rehydrated before staining with haematoxylin and eosin. The stained sections were examined under a light microscope for evaluation of the structural integrity of the tongue mucosa. Histopathological assessment focused on epithelial integrity, epithelial degeneration or ulceration, inflammatory-cell infiltration, oedema,

vascular changes, connective-tissue alterations and other pathological features associated with methotrexate-induced oral mucosal injury. The histological findings of the disease-control group were compared with those of the normal, standard-treatment and F3-treated groups. Preservation of epithelial architecture, reduction in inflammatory changes and restoration of normal tissue organization in the F3-treated group were considered supportive of the protective or therapeutic effect of the herbal gargle.

Statistical Analysis

The experimental observations were expressed as mean $\pm$ standard error of the mean (SEM), where applicable. The results obtained from the different experimental groups were statistically compared using an appropriate statistical test, such as one-way analysis of variance (ANOVA), followed by a suitable post-hoc multiple-comparison test. The choice of statistical test was based on the experimental design and number of groups being compared. Differences between groups were considered statistically significant at the predetermined level of significance, generally $p < 0.05$. The statistical analysis was performed using suitable statistical software.

3. Results

Table 1. Percentage inhibition of carrageenan-induced paw edema

Treatment	Dose	1 h	2 h	3 h	4 h
Control	—	1.73	1.62	1.59	1.51
EAM	100 mg/kg	26.23%	28.03%	34.22%	50.32%
EAM	200 mg/kg	32.32%	35.01%	50.15%	62.21%
EPG	100 mg/kg	24.23%	28.03%	39.22%	49.32%
EPG	200 mg/kg	30.32%	32.01%	51.15%	65.21%
Indomethacin	10 mg/kg	46.23%	57.45%	69.32%	80.11%

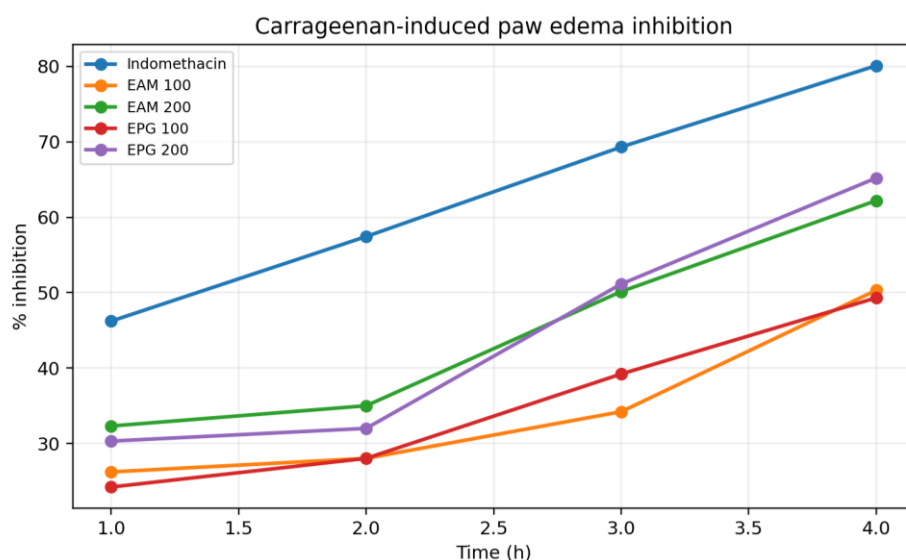
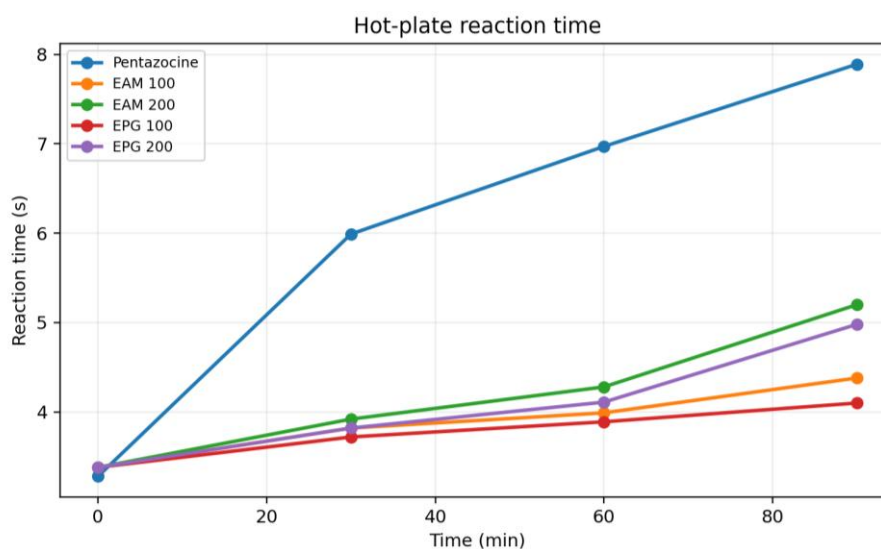


Figure 1. Time-dependent inhibition of carrageenan-induced paw edema.

Table 2. Hot-plate reaction time (s)

Treatment	0 min	30 min	60 min	90 min
Control	3.28	3.66	3.62	3.20
Pentazocine 100 mg/kg	3.28	5.99	6.97	7.89
EAM 100 mg/kg	3.38	3.82	3.99	4.38
EAM 200 mg/kg	3.38	3.92	4.28	5.20
EPG 100 mg/kg	3.38	3.72	3.89	4.10
EPG 200 mg/kg	3.38	3.82	4.11	4.98

**Figure 2.** Time-dependent change in hot-plate reaction time.**Table 3.** Physicochemical evaluation of herbal gargles

Formulation	pH	Viscosity (cP)	Specific gravity	Total solids (%)
F1	6.24 ± 0.02	9.02	1.035	16.08
F2	6.37 ± 0.03	9.38	1.038	16.21
F3	6.45 ± 0.04	9.64	1.041	16.36
F4	6.38 ± 0.05	9.46	1.039	16.27
F5	6.28 ± 0.06	9.05	1.037	16.15

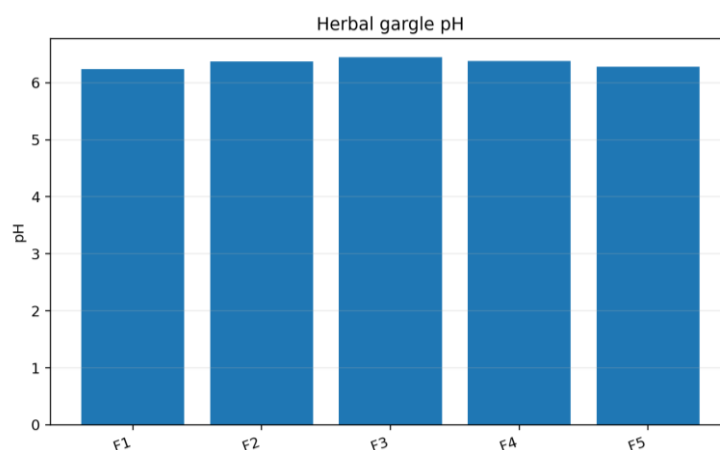
**Figure 3.** pH profile of the five herbal gargle formulations.

Table 4. Microbial load of herbal gargles

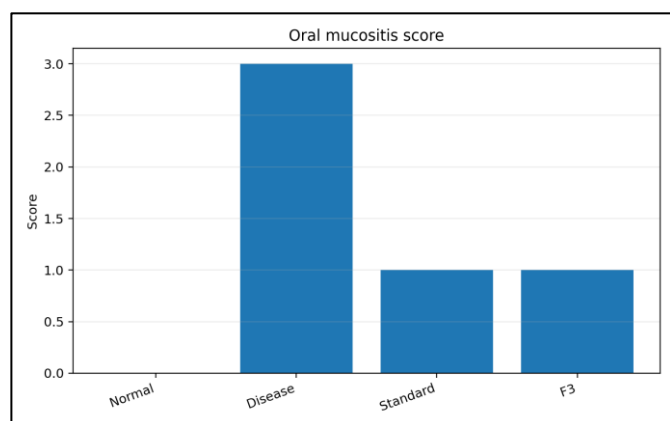
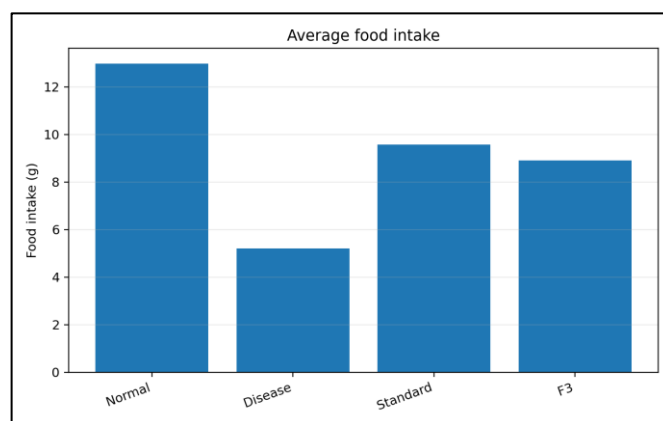
Formulation	Aerobic count (CFU/mL)	Fungal count (CFU/mL)	Overall quality
F1	32	7	Good
F2	28	5	Good
F3	18	3	Excellent
F4	31	6	Good
F5	35	7	Good

Table 5. Body-weight changes in methotrexate-induced oral mucositis

Group	Initial BW (g)	Final BW (g)	Weight change (g)
Normal control	200.5 ± 17.30	240.0 ± 12.21	40 ± 10.06
Disease control	210.5 ± 17.30	190.5 ± 12.21	-20 ± 10.06
Standard treated	220.7 ± 18.70	254.0 ± 16.27	34 ± 11.24
Herbal gargle F3	231.7 ± 18.70	251.7 ± 16.27	20 ± 11.24

Table 6. Functional and clinical outcomes in the oral mucositis model

Group	Average food intake (g)	OMS	Mortality day 7	Mortality day 14
Normal control	12.98 ± 1.25	0	0%	0%
Disease control	5.21 ± 0.38	3	50%	100%
Standard treated	9.58 ± 0.51	1	0%	0%
Herbal gargle F3	8.91 ± 0.42	1	0%	0%

**Figure 4.** Oral mucositis score in the four experimental groups.**Figure 5.** Average food intake in the four experimental groups.**Table 7.** Hematological parameters during oral mucositis

Group	WBC day 7	RBC day 7	PLT day 7	WBC day 12	RBC day 12	PLT day 12
Normal	15.8 ± 0.52	7.76 ± 0.12	565.3 ± 11.67	16.14 ± 0.52	7.94 ± 0.22	578.2 ± 16.43
Disease	3.2 ± 1.24	5.86 ± 0.19	161.20 ± 27.01	3.1 ± 1.20	7.10 ± 0.19	172.33 ± 30.02
Standard	14.52 ± 1.21	6.34 ± 0.28	267.21 ± 99.70	14.85 ± 1.29	5.42 ± 0.28	510.21 ± 78.70
F3	13.89 ± 2.23	6.94 ± 0.71	245.33 ± 67.17	13.79 ± 2.36	5.51 ± 0.71	414.33 ± 67.17

4. Discussion

The carrageenan results demonstrate that both ethanolic extracts reduced acute inflammatory edema in a dose- and time-dependent manner. EAM 200 mg/kg produced 62.21% inhibition at 4 h, whereas EPG 200 mg/kg produced 65.21%. The reference indomethacin reached 80.11%. The stronger response at 3-4 h is consistent with the work interpretation that the extracts were effective during the later phase of carrageenan inflammation, although this model cannot reproduce the full pathogenesis of cancer-treatment-induced mucositis. The hot-plate data provide a second pharmacological dimension. EAM 200 mg/kg increased reaction time from 3.38 s at baseline to 5.20 s at 90 min, while EPG 200 mg/kg reached 4.98 s. Both were less active than pentazocine, which reached 7.89 s. The dose-response pattern supports analgesic potential but should not be interpreted as proof of a central mechanism because the hot-plate model is a screening model rather than an oral-mucosa-specific pain model. Formulation evaluation identified F3 as the most balanced preparation. Its pH was 6.45, viscosity 9.64 cP, specific gravity 1.041 and total solids 16.36%. The source work reports F3 as having very slight sedimentation, excellent redispersibility and low/minimal foam formation. These characteristics are important for a gargle because the preparation must remain pourable and easily dispersed while maintaining adequate contact with oral mucosa.

Stability testing further supported F3. At room temperature, its pH changed from 6.45 to 6.42 without significant appearance change or phase separation. Under accelerated conditions, pH changed from 6.45 to 6.36, again without significant appearance change or phase separation. F3 also had the lowest aerobic microbial count (18 CFU/mL) and fungal count (3 CFU/mL) among F1-F5. The source work consequently classified F3 as very stable and microbiologically excellent. The oral mucositis experiment provided the most clinically relevant preclinical evidence. The disease-control animals lost approximately 20 g over the study period, while the normal, standard and F3 groups gained weight. Average food intake was markedly reduced in disease control to 5.21 g compared with 12.98 g in normal controls; F3-treated animals showed 8.91 g. These findings indicate partial recovery of nutritional intake following treatment.

The mucositis score changed from 3 in disease control to 1 in the standard and F3 groups, while normal controls had a score of 0. Thus, F3 was associated with a marked reduction in visible mucosal injury. The mortality pattern was also notable: disease control reached 100% mortality by day 14, whereas normal, standard and F3 groups had 0% mortality. The work therefore describes F3 as protective under the experimental conditions. Hematological findings provide additional supportive evidence. On day 7, disease control showed marked reductions in WBC and platelet counts compared with normal controls, whereas standard and F3 groups showed higher WBC and platelet values. By day 12, the standard and F3 groups

showed substantial recovery of platelet counts, although RBC values remained lower than normal in the treatment groups. These data should be interpreted descriptively because the article does not introduce new statistical testing beyond the work-reported significance statements. Histopathology strengthened the functional observations. Normal control tongues showed intact stratified squamous epithelium and preserved connective tissue. Disease control displayed epithelial disruption, erosion, ulcerative changes, inflammatory-cell infiltration and edema. Standard treatment improved epithelial continuity and reduced inflammatory changes. F3 showed comparatively intact and better organized epithelium, reduced erosion and inflammatory infiltration, and improved connective-tissue integrity. The integrated findings suggest a plausible multi-target profile. The antioxidant work indicates that EAM and EPG can neutralize several reactive radical species; the anti-haemolytic assay suggests membrane protection; carrageenan testing demonstrates anti-inflammatory potential; the hot-plate test indicates analgesic screening activity; and the F3 gargle was associated with improved clinical and histological outcomes in the oral mucositis model. The work attributes these effects broadly to phenolics, flavonoids, tannins, terpenoids and other bioactive constituents. Importantly, this is a pharmacological interpretation rather than proof that any one compound or molecular pathway caused the observed effects. An important methodological issue should be transparently retained from the source. The composition table in the methods section states a total herbal extract of 10 g/100 mL, whereas a later selection statement describes F3 as containing a total herbal extract concentration of 5 g/100 mL. Because the experimental formulation table explicitly reports 10 g/100 mL, this article uses the tabulated composition as the primary formulation record and recommends resolving the discrepancy before journal submission. This is preferable to silently changing the work data.

The body-weight data similarly show a clear disease-associated effect. Normal animals gained 40 g, while disease-control animals lost 20 g. Standard-treated animals gained 34 g and F3-treated animals gained 20 g. Thus, F3 improved the overall trajectory but did not reproduce the full weight gain of the normal or standard groups. This nuance should be preserved in a research article because it prevents overstatement of efficacy.

The food-intake data are particularly meaningful because oral mucositis is not simply a visible lesion. Pain, ulceration and inflammation can make eating difficult and can lead to nutritional compromise. The increase from 5.21 g in disease control to 8.91 g in F3 therefore represents a functional outcome that complements the mucositis score. Although F3 did not restore intake completely to the normal-control value of 12.98 g, the direction of change was favorable.

The methotrexate-induced oral mucositis findings provide the most direct experimental connection. Disease-control animals showed substantial deterioration in body weight, food intake, mucositis

score and survival. In contrast, the F3 group gained 20 g during the study, consumed 8.91 g of food on average and showed a mucositis score of 1 compared with 3 in disease control. The absence of mortality in F3 was also notable. These outcomes collectively indicate improvement rather than an isolated change in one parameter.

The stability data further strengthened the selection. F3 showed only a small pH change under room-temperature storage and under accelerated conditions, with no significant appearance change and no phase separation. The microbial counts were also the lowest among the tested formulations. The source work consequently selected F3 as the optimized preparation and reported favorable antimicrobial and in-vitro anti-mucositis performance. These combined criteria are more informative than selecting a formulation on one endpoint.

The organoleptic results also favored F3. The formulation was described as greenish brown, clear to slightly opalescent, with characteristic peppermint odor, mild acceptable taste, very good homogeneity and absence of phase separation. By comparison, F5 was described as bitter and showed moderate foam persistence. This demonstrates that formulation optimization should not be based on biological activity alone. Palatability, appearance, physical uniformity and handling characteristics can strongly influence acceptability of a mouth-rinse product.

F3 represented the middle formulation in the ratio series and contained equal proportions of the two ethanolic extracts. The physicochemical results suggest that this balance was favorable. Its pH of 6.45 was the highest among the five formulations but remained close to the other values. Its viscosity of 9.64 cP was also the highest, yet the difference from the other formulations was small. The combination of acceptable viscosity, excellent redispersibility and low foam formation is practically important because a gargle must be easy to dispense, rinse and expel.

In the hot-plate assay, the higher extract dose again produced the greater response. EAM 200 mg/kg reached 5.20 s at 90 min and EPG 200 mg/kg reached 4.98 s, compared with 7.89 s for pentazocine. The response was therefore moderate relative to the standard. For oral mucositis, analgesic activity is potentially useful because pain is a major determinant of reduced food and fluid intake. However, local oral pain may involve inflammatory and peripheral mechanisms that are not fully represented by a thermal nociception model.

The EAM response in the carrageenan model increased from 50.32% inhibition at 4 h with 100 mg/kg to 62.21% with 200 mg/kg. EPG showed a similar dose effect, increasing from 49.32% to 65.21%. The reference indomethacin reached 80.11%. Because carrageenan inflammation develops through sequential mediator release, the stronger later-phase inhibition is of interest, but it does not establish a particular molecular target.

The results can reasonably be described as evidence of acute anti-inflammatory potential.

The pharmacological findings can be viewed as a sequence from extract activity to dosage-form performance and finally to an oral mucositis model. The carrageenan experiment demonstrated that EAM and EPG reduced acute inflammatory edema, while the hot-plate experiment demonstrated increased reaction latency. These findings were not identical in magnitude, and neither extract equaled the corresponding standard drug. Their value lies in demonstrating complementary pharmacological properties that may be relevant to the inflammatory and painful components of mucositis. Histopathology provided structural confirmation of the clinical findings. Disease-control tongues showed epithelial erosion, ulcerative changes, inflammatory infiltration and edema, whereas F3-treated tongues showed improved epithelial continuity and reduced inflammatory changes. Histological restoration is important because it provides tissue-level evidence that complements the numerical mucositis score. The work interprets the improvement as compatible with antioxidant, anti-inflammatory, mucosal-protective and tissue-repairing actions of the combined extracts.

The hematological findings suggest systemic consequences of the mucositis-induction protocol and partial recovery after treatment. Disease-control WBC and platelet values were substantially lower than normal on day 7. Standard and F3 groups showed higher WBC and platelet values than disease control. By day 12, platelet recovery was especially pronounced in the standard group and substantial in F3. These observations are supportive but should be interpreted cautiously because the experimental design and sample-size details available in the source do not justify adding new inferential statistics.

5. Conclusion

Ethanolic extracts of *A. millefolium* and *P. guajava* showed dose- and time-dependent anti-inflammatory and analgesic screening activity. Their incorporation into herbal gargles produced formulations with acceptable physicochemical properties, and F3 showed the most favorable overall formulation profile. In the methotrexate-induced oral mucositis model, F3 was associated with improved body-weight trajectory, higher food intake, lower mucositis score, absence of mortality, partial hematological recovery and substantial restoration of tongue histology. Taken together, the findings support F3 as a promising preclinical polyherbal gargle candidate for further investigation in oral mucositis. The results remain supportive rather than definitive, and clinical efficacy, safety, standardized phytochemical markers and interaction with anticancer therapy must be established before therapeutic use can be recommended.

Acknowledgement: The authors gratefully acknowledge the support and facilities provided by Ram Krishna Dharmarth Foundation University, Gandhi Nagar, Bhopal, M.P., which contributed to the successful

completion of this research work and preparation of the present manuscript.

Author's contribution: Priyanka Gupta contributed to the conceptualization of the study, literature review, experimental work, data collection, data analysis, and preparation of the initial manuscript. Prof. M. L. Kori provided supervision and guidance throughout the study, contributed to the interpretation and critical evaluation of the results, and reviewed and approved the final manuscript. Both authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Funding source: Not applicable

Conflicts of interest: There is no conflict of interest

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