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Development and evaluation of an antiseptic nasal spray based on aqueous extract of *Ocimum gratissimum* L.: Formulation, phytochemical characterization and antimicrobial activity

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Abstract

Introduction: Upper respiratory tract infections (URTIs) are common and frequently treated with antibiotics, contributing to the emergence of bacterial resistance. Medicinal plant extracts represent a promising natural alternative. *Ocimum gratissimum*, rich in bioactive secondary metabolites, has noteworthy antiseptic potential.

Methods: Leaves of *O. gratissimum* were extracted by aqueous infusion and phytochemically characterized. Antimicrobial activity was evaluated in vitro against Staphylococcus aureus using broth microdilution with resazurin to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Nine nasal spray formulations were developed, physicochemically evaluated, and subjected to microbiological quality control according to the European Pharmacopoeia.

Results: The extraction yield was 33.33%. Phytochemical screening revealed the presence of polyphenols, flavonoids, saponins, alkaloids, sterols, terpenes, and cardiotonic glycosides. Total flavonoid and polyphenol contents were 1413 ± 0.836 mg QE/g dry extract and 202.098 ± 0.691 mg GAE/g dry extract, respectively. The extract exhibited bacteriostatic activity against *S. aureus* (MIC = 0.048 mg/mL; MBC = 0.195 mg/mL; MBC/MIC ≥ 4). The final formulation showed a pH compatible with the nasal mucosa, consistent spray volume per actuation, and compliance with microbiological standards.

Conclusion: The formulated nasal spray containing aqueous extract of *O. gratissimum* is stable, safe, and demonstrates promising *in vitro* antimicrobial activity. Further tolerance and clinical efficacy studies are required before therapeutic application.

Keywords: *Ocimum gratissimum*; Nasal Spray; Aqueous Extraction; Antimicrobial Activity; Pharmaceutical Formulation.

1. Introduction

Upper respiratory tract infections (URTIs) are among the most frequent reasons for medical consultation and are predominantly viral in origin. The systematic prescription of antibiotics contributes to the emergence of bacterial resistance, highlighting the need for effective and safe local alternatives.

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Ocimum gratissimum (Lamiaceae) is widely used in traditional medicine to treat respiratory and digestive infections. Extracts of this plant contain flavonoids, polyphenols, essential oils, and other secondary metabolites known for their antimicrobial, antioxidant, and anti-inflammatory activities [1–5].

Few studies have explored its incorporation into a stable nasal formulation that complies with pharmaceutical and microbiological standards. The objective of this study was to develop and evaluate an antiseptic nasal spray based on aqueous extract of *O. gratissimum*.

Figure 1 *Ocimum gratissimum* L (present study).

2. Materials and Methods

2.1. Type and Study Period

This experimental study was conducted over seven months, from November 4, 2024 to May 30, 2025, ensuring comprehensive monitoring of extraction, formulation, and evaluation stages.

2.2. Plant Material

Fresh leaves of *O. gratissimum* were collected in Zamengoé (Okola, Cameroon) under controlled ecological conditions to prevent contamination. Botanical authentication was confirmed at the National Herbarium of Cameroon. Leaves showing mold or deterioration were excluded.

2.3. Extraction

After drying at 50°C for 48 hours, the leaves were ground into fine powder to increase contact surface with water and maximize extraction of soluble compounds. Infusion was carried out in boiling distilled water with gentle stirring for 30 minutes to efficiently extract hydrosoluble metabolites. The extract was filtered using Whatman No. 1 filter paper and concentrated under controlled temperature evaporation to prevent degradation of thermosensitive compounds. Extraction yield was calculated as a percentage of the initial powdered mass.

2.4. Phytochemical Characterization

Qualitative screening was performed to detect alkaloids, flavonoids, saponins, polyphenols, sterols, terpenes, and cardiotonic glycosides.

Total polyphenols were quantified using the Folin–Ciocalteu method and expressed as mg gallic acid equivalent (GAE)/g dry extract, while total flavonoids were measured by aluminum chloride complexation and expressed as mg quercetin equivalent (QE)/g dry extract. All measurements were performed in triplicate to ensure reproducibility.

2.5. Evaluation of Antimicrobial Activity

Antibacterial activity was evaluated against *Staphylococcus aureus* CIP 7625 using broth microdilution with resazurin as a growth indicator. The inoculum was standardized to 1.5×10^8 CFU/mL. MIC and MBC were determined after 24 hours of incubation at 37°C. Gentamicin served as positive control, and a sterility control was included. The MBC/MIC ratio was used to characterize bacteriostatic or bactericidal action.

2.6. Nasal Spray Formulation

The dried extract was incorporated into an aqueous phase containing excipients selected for nasal mucosa compatibility. Carboxymethylcellulose (CMC) was used as a viscosity-enhancing agent, while sorbitol and xylitol ensured isotonicity and mucosal comfort. Polysorbate 20 improved homogeneity and dispersion. Preservatives and a buffering system were added to maintain pH between 5.5 and 6.5, promoting mucosal tolerance.

Nine formulations were tested and evaluated for viscosity, physical stability, spray continuity, and delivered volume per actuation.

2.7. Microbiological Control

The final product underwent microbiological quality control according to the European Pharmacopoeia (11th edition, 2023). Total aerobic microbial count and yeast/mold counts were determined using selective media (PCA, Sabouraud, MacConkey, Chapman, and cetrimide agar). Absence of *E. coli*, *S. aureus*, and *P. aeruginosa* was verified.

2.8. Statistical Analysis

Data were expressed as mean ± standard deviation (n = 3) and analyzed using Microsoft Excel.

3. Results

3.1. Extraction Yield

The yield of the aqueous extraction was 33.33%, indicating that one third of the initial mass of leaf powder was recovered in the form of soluble metabolites. This yield is consistent with data reported in the literature and suggests an efficient extraction of hydrosoluble compounds.

3.2. Phytochemical Profile

Qualitative phytochemical screening confirmed the presence of several classes of bioactive compounds, including polyphenols, flavonoids, saponins, alkaloids, sterols, terpenes, and cardiotonic glycosides.

Quantitative assays showed that the flavonoid content was 1413 ± 0.836 mg quercetin equivalent per gram of dry extract, while the total polyphenol content was 202.098 ± 0.691 mg gallic acid equivalent per gram of dry extract. These high concentrations confirm the pharmacological potential of the extract and justify its incorporation into a topical formulation.

3.3. Antimicrobial Activity

The aqueous extract exhibited a minimum inhibitory concentration (MIC) of 0.048 mg/mL and a minimum bactericidal concentration (MBC) of 0.195 mg/mL against Staphylococcus aureus, indicating a bacteriostatic effect (MBC/MIC = 4). This activity supports the traditional use of the plant and confirms its in vitro antiseptic potential.

3.4. Formulation and Quality Control

Among the nine formulations tested, the final selected formulation is presented in Table I.

Table 1 Composition of the selected formulation

Ingredients	Extract *O. gratissumum*	Carboxymethyl-cellulose	Sorbitol	Tween 20	Phosphate de sodium	Purified Water
Quantity (mg)	20	1	3,5	0,30	1	q.s. 100 mL

This formulation was selected because it demonstrated a pH compatible with the nasal mucosa (approximately 5.5–6.5), homogeneous and continuous spray performance, a constant volume delivered per actuation, and satisfactory physical stability without sedimentation or color change.

Figure 2 Nasal spray bottles (present study).

3.5. Microbiological Quality Control of the Finished Product

The formulated nasal spray complied with the microbiological limits defined by the European Pharmacopoeia. The results confirmed the absence of contamination by *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and demonstrated that total microbial counts as well as yeast and mold counts were within the permitted limits.

4. Discussion

The aqueous extract of *Ocimum gratissimum* demonstrated a rich and diverse phytochemical profile, including flavonoids, polyphenols, saponins, alkaloids, sterols, terpenes, and cardiotonic glycosides. These secondary metabolites are recognized for their antimicrobial, antioxidant, and anti-inflammatory properties, which corroborates the bacteriostatic activity observed against Staphylococcus aureus [1–3].

The measured concentrations (flavonoids: 1413 ± 0.836 mg QE/g dry extract; polyphenols: 202.098 ± 0.691 mg GAE/g dry extract) are significant and suggest that the extract may exert a local pharmacological effect when administered intranasally. Flavonoids and polyphenols are known to disrupt bacterial membranes, inhibit enzymatic synthesis, and reduce microbial adhesion, which may explain the observed bacteriostatic mechanism [4,5].

The MIC (0.048 mg/mL) and MBC (0.195 mg/mL) values obtained are consistent with literature data on aqueous extracts of *O. gratissimum* [4]. The MBC/MIC ratio of 4 indicates a bacteriostatic effect, suggesting that the spray could limit bacterial proliferation on the nasal mucosa without completely eliminating the local flora, thereby reducing the risk of microbial imbalance.

The final nasal spray formulation exhibited a pH compatible with the nasal mucosa (approximately 5.5–6.5), homogeneous and continuous spray performance, and a constant volume delivered per actuation. These physicochemical criteria are essential to ensure safe and effective administration. Microbiological quality control

confirmed the absence of contamination by pathogenic bacteria and compliance with the limits established by the European Pharmacopoeia, ensuring the product's safety for topical use [6].

Study Limitations

Antimicrobial activity was evaluated only against *S. aureus*. Other bacteria responsible for upper respiratory tract infections, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, should be tested to confirm a broader spectrum of activity.

- Mucosal tolerance was not assessed through cytotoxicity assays or in vivo models, which constitutes an essential step prior to clinical trials.
- Long-term stability has not yet been evaluated. Accelerated and real-time stability studies are required to determine the optimal shelf life.

Perspectives

- Conduct additional tests on a broader panel of nasal pathogens.
- Evaluate local tolerance using cellular and animal models before clinical trials.
- Investigate combined formulations with other natural extracts to explore potential synergistic effects.
- Integrate this spray into strategies aimed at reducing systemic antibiotic use in recurrent upper respiratory tract infections, thereby helping to limit the emergence of bacterial resistance.

In summary, the aqueous extract of *O. gratissimum*, combined with an appropriate pharmaceutical formulation, represents a promising natural alternative to synthetic antiseptic sprays, offering in vitro efficacy together with pharmaceutical safety.

5. Conclusion

This study demonstrates that aqueous extract of *Ocimum gratissimum* can be successfully incorporated into a stable, microbiologically safe nasal spray compatible with nasal mucosa. The extract exhibits significant *in vitro* antimicrobial activity against *Staphylococcus aureus*, suggesting promising antiseptic potential for topical management of upper respiratory tract infections. Further tolerance, *in vivo* efficacy, and clinical studies are necessary to confirm its therapeutic applicability.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

They further declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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