

Antibacterial Activity of Red Onion and White Ginger Extract Combinations Against *Staphylococcus aureus* In Vitro

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ABSTRACT

Rhinosinusitis is an inflammatory disease of the nose and paranasal sinuses in which *Staphylococcus aureus* is a frequent causative pathogen. Rising antibacterial resistance has prompted interest in plant-derived agents such as red onion and white ginger, which contain flavonoids, alkaloids, saponins, terpenoids, and essential oils with reported antimicrobial activity. This study evaluated the in vitro antibacterial activity of combined red onion (*Allium cepa* L.) and white ginger (*Zingiber officinale* Roscoe) ethanol extracts against *S. aureus*. Extracts were prepared in 96% ethanol by maceration and combined at ratios of 25%:75%, 50%:50%, and 75%:25% (red onion:white ginger); 100% single extracts were also tested. Antibacterial activity was assessed using the agar well diffusion method on Mueller-Hinton agar seeded with *S. aureus* (0.5 McFarland). Ciprofloxacin 50 µg/mL and dimethyl sulfoxide (DMSO) served as positive and negative controls, respectively. Inhibition zones were measured in millimetres and analysed using one-way ANOVA followed by the Games-Howell test. The extract combinations produced mean inhibition zones ranging from 2.98 to 7.80 mm. The 75%:25% combination yielded the largest inhibition zone (7.80 ± 0.26 mm), whereas the 25%:75% combination produced the smallest (2.98 ± 1.71 mm). Ciprofloxacin generated a markedly larger inhibition zone (29.50 ± 1.32 mm), while DMSO showed no inhibition. Statistical analysis showed significant differences between most extract combinations and the controls ($p < 0.05$), except for the 25%:75% combination versus the negative control. In conclusion, combined red onion and white ginger ethanol extracts exhibit modest in vitro antibacterial activity against *S. aureus*, with the 75%:25% ratio being the most active, although still far weaker than ciprofloxacin.



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Keywords:

Antibacterial activity; *Allium cepa* extract; *Zingiber officinale* extract; *Staphylococcus aureus*; Agar well diffusion method

Received:
2025-07-15

Accepted:
2025-12-17

Online:
2025-12-18

1. Introduction

Rhinosinusitis (RS) is an inflammatory condition involving the nose and paranasal sinuses that is classified according to symptom duration into acute (< 12

weeks) and chronic (≥ 12 weeks). Clinical manifestations of RS include nasal congestion, mucopurulent nasal discharge, facial pain, and olfactory disturbances [1],[2]. Based on data from the National Health Interview Survey, rhinosinusitis is one of the most common causes of health-care visits in the United States within a one-year period, with approximately 20 million cases of acute bacterial rhinosinusitis occurring annually [1]. A study conducted at dr. Zainoel Abidin General Hospital, Banda Aceh, in 2019–2020 reported 445 patients diagnosed with rhinosinusitis, of whom 60.30% had acute and 39.70% had chronic rhinosinusitis [3].

Staphylococcus aureus is part of the normal flora of the upper airway but is also a frequent pathogen responsible for rhinosinusitis [4]. *S. aureus* is a Gram-positive bacterium with strong environmental resistance and can grow well at temperatures of 18–40 °C [5]. Increasing antimicrobial resistance among *S. aureus* strains has driven interest in identifying alternative or adjunctive antibacterial agents derived from medicinal plants.

Red onion (*Allium cepa* L.) is a seasonal herbaceous plant with a layered bulb that has long been used traditionally to treat conditions such as fever and influenza. Previous studies have shown that red onion possesses antibacterial properties, which are attributed to bioactive compounds including flavonoids, alkaloids, saponins, and allicin [6]. White ginger (*Zingiber officinale* Roscoe) is widely used as a spice and traditional medicine. Its rhizomes contain secondary metabolites such as flavonoids, phenols, terpenoids, and essential oils that have been reported to inhibit various bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* [7].

Several studies have evaluated combinations of plant extracts containing similar phytochemicals. Harlita et al. reported that combinations of dayak onion and red ginger ethanol extracts produced inhibition zones of 8.00–9.89 mm against *Salmonella typhi* at various ratios [8]. Pohan et al. demonstrated that red ginger and kencur extract combinations yielded the largest inhibition zone (11.4 mm) at 100% red ginger extract against *S. aureus* [9]. Azkiyah reported that ginger rhizome extract contains essential oils, flavonoids, phenols, and terpenoids, which can inhibit bacterial growth by targeting topoisomerase II (DNA gyrase) and other enzymes in Gram-positive bacteria including *S. aureus* [10].

Based on previous evidence of the antibacterial activity of red onion and ginger extracts, this study aimed to evaluate the in-vitro antibacterial effect of a combination of red onion (*Allium cepa* L.) and white ginger (*Zingiber officinale* Roscoe) ethanol extracts against *Staphylococcus aureus*.

2. Methods

Study design and setting

This research was an in-vitro experimental study using a post-test-only control group design. The study was conducted at the Microbiology and Parasitology Laboratory and the Chemistry and Natural Materials Laboratory of Abdurrah University, using a combination of red onion and white ginger extracts tested against *Staphylococcus aureus*.

Materials

Red onion, white ginger, *Staphylococcus aureus* culture, Mueller–Hinton agar (MHA), nutrient agar (NA), 1% barium chloride (BaCl_2) solution, DMSO, 1% sulfuric acid (H_2SO_4), ciprofloxacin 500 mg, 1% ferric chloride (FeCl_3), distilled water,

hydrochloric acid (HCl), potassium permanganate (KMnO₄), magnesium (Mg), 0.9% sodium chloride (NaCl), and 96% ethanol were used in this study.

Preparation of red onion and white ginger extracts

Fresh red onions and white ginger rhizomes (1 kg each) were washed with running water, thinly sliced, and dried in an oven. After drying, 100 g of each sample was transferred into a glass bottle and macerated with 1000 mL of 96% ethanol for 3 × 24 h with occasional stirring. The macerates were filtered and concentrated using a rotary evaporator at 50 °C to obtain thick ethanol extracts. Phytochemical screening of red onion and white ginger extracts was then conducted to identify the presence of flavonoids, alkaloids, saponins, terpenoids, and essential oils [8].

Preparation of extract concentrations and combinations

The crude ethanol extracts (100%) of red onion and white ginger were diluted with DMSO to obtain 25%, 50%, and 75% concentrations. These were then combined to produce three mixtures with the following ratios of red onion:white ginger: 25%:75%, 50%:50%, and 75%:25% [8]. In addition, 100% red onion extract and 100% white ginger extract were tested individually.

Rejuvenation and confirmation of *Staphylococcus aureus*

Staphylococcus aureus was rejuvenated on nutrient agar (NA) and incubated at 37 °C for 24 h. Macroscopic observation of colony growth was carried out to assess morphology. A loopful of culture was placed on a glass slide, fixed, and Gram-stained according to the procedure of Cappuccino and Welsh [11]. Microscopic observation confirmed the presence of purple, Gram-positive cocci arranged in irregular clusters.

Preparation of *Staphylococcus aureus* suspension

Bacteria were transferred into a tube containing 2 mL of 0.9% NaCl using an inoculating loop and homogenised until a visibly turbid suspension formed. Turbidity was adjusted to match the 0.5 McFarland standard, corresponding to approximately 10⁸ CFU/mL [10].

Antibacterial activity test of extract combinations

Antibacterial activity was evaluated using the well diffusion method. MHA plates were inoculated with the standardised *S. aureus* suspension. Wells were made in the agar using a sterile cork borer to create seven wells per plate, which were filled with: positive control (ciprofloxacin 50 µg/mL), negative control (DMSO), 100% red onion extract, 100% white ginger extract, and the three extract combinations (25%:75%, 50%:50%, 75%:25%). Plates were incubated at 37 °C for 24 h. After incubation, inhibition zones around each well were measured in millimetres using a vernier caliper [8],[9].

Data analysis

The data obtained were analysed using SPSS 25.0. One-way ANOVA was used to determine significant differences among treatment groups, followed by the Games-Howell post hoc test. A p-value < 0.05 was considered statistically significant.

3. Results and Discussion

This study generated data on the phytochemical content of red onion and white ginger ethanol extracts, confirmation of the *Staphylococcus aureus* test strain, and the antibacterial activity of extract combinations as reflected by inhibition zone diameters.

Phytochemical Screening

Phytochemical screening of red onion and white ginger ethanol extracts is presented in **Table 1**. Red onion extract was positive for flavonoids, alkaloids, and saponins, whereas white ginger extract contained flavonoids, terpenoids, and essential

oils. These findings are consistent with previous studies reporting that red onion bulbs contain flavonoids and sulfur compounds with antibacterial activity, while ginger rhizomes are rich in phenolic compounds and volatile oils [6],[7]. The presence of overlapping classes of secondary metabolites, particularly flavonoids, suggests a potential for combined or synergistic effects when the extracts are used together.

No	Phytochemical test	Red onion extract	White ginger extract
1	Flavonoids	+	+
2	Alkaloids	+	-
3	Saponins	+	-
4	Terpenoids	-	+
5	Essential oils	-	+

Note: +: present; -: not detected

Flavonoids may interfere with bacterial growth by forming complexes with extracellular proteins and cell wall components, disrupting membrane integrity, and inhibiting DNA gyrase and nucleic acid synthesis [6]. Alkaloids can inhibit peptidoglycan synthesis in the bacterial cell wall, resulting in impaired structural integrity and cell lysis [12]. Saponins are known to increase membrane permeability through interaction with membrane sterols, leading to leakage of intracellular constituents [6]. Terpenoids and essential oils further disrupt membrane function by altering membrane fluidity and damaging porin proteins, thereby reducing nutrient uptake and causing cell death [13],[14]. The collective action of these compounds is likely responsible for the antibacterial effects observed in this study.

Confirmation of *Staphylococcus aureus*

Macroscopic examination of the rejuvenated bacterial culture on nutrient agar showed white, opaque, circular colonies with smooth edges, consistent with typical *Staphylococcus* morphology. Gram staining revealed purple, Gram-positive cocci arranged in irregular clusters, confirming the identity of *S. aureus* as described in standard microbiology references [5],[11]. Confirmation of the test strain is important to ensure the validity of subsequent antibacterial testing.

Inhibition zones of extract combinations

The antibacterial activity of the combined red onion and white ginger ethanol extracts against *Staphylococcus aureus* was assessed by measuring inhibition zones around the wells on Mueller–Hinton agar plates. The qualitative appearance of the inhibition zones for each treatment – three extract combinations, 100% extracts, and the positive and negative controls – is illustrated in **Figure 1**, while the quantitative data are summarised in **Table 2**.

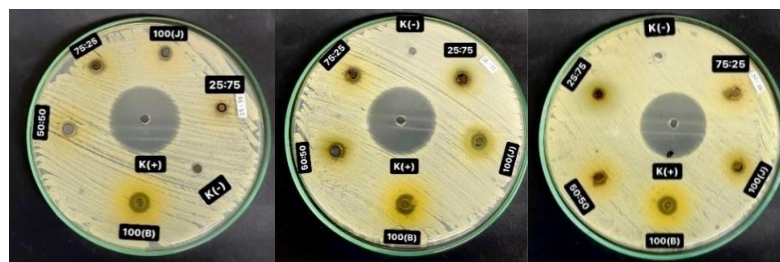


Figure 1. Inhibition zones of red onion-white ginger ethanol extracts and their combinations against *Staphylococcus aureus*. K(+), ciprofloxacin (positive control); K(-), DMSO (negative control); 100(B), 100% red onion; 100(J), 100% white ginger; 25:75, 50:50, 75:25, red onion:white ginger (v/v)

As presented in **Table 2**, all three extract combinations produced measurable inhibition zones against *S. aureus*, with mean diameters ranging from 2.98 to 7.80 mm. The smallest mean inhibition zone was observed for the 25%:75% red onion:white ginger combination (2.98 ± 1.71^a mm), indicating relatively weak antibacterial activity. Increasing the proportion of red onion to a 50%:50% ratio resulted in a larger mean inhibition zone (5.32 ± 0.75^b mm), classified as moderate inhibition. The highest proportion of red onion (75%:25%) yielded the largest mean inhibition zone among the extract combinations (7.80 ± 0.26^c mm), suggesting that higher red onion content enhances the antibacterial effect. According to the Davis and Stout inhibition criteria, the 25%:75% combination falls within the weak category, whereas the 50%:50% and 75%:25% combinations are in the moderate category.

The control treatments showed the expected pattern. The negative control (DMSO) produced no inhibition (0.00 ± 0.00^a mm), confirming that the solvent lacked intrinsic antibacterial activity. In contrast, the positive control (ciprofloxacin) generated a much larger inhibition zone (29.50 ± 1.32^d mm), consistent with its established potency as a broad-spectrum antibacterial agent. The substantial difference between ciprofloxacin and all extract combinations indicates that, under the conditions of this assay, the plant extracts exhibit only modest activity compared with standard antibiotic therapy.

Table 2. Inhibition zones of red onion-white ginger extract combinations against *Staphylococcus aureus*

Treatment (red onion : white ginger)	n	Range (min-max), mm	Mean \pm SD, mm
25% : 75%	3	1.71–4.95	2.98 ± 1.71^a
50% : 50%	3	4.50–6.00	5.32 ± 0.75^b
75% : 25%	3	7.50–8.00	7.80 ± 0.26^c
Control (+), ciprofloxacin	3	28.00–30.50	29.50 ± 1.32^d
Control (-), DMSO	3	0.00–0.00	0.00 ± 0.00^a

Note: Values are presented as mean \pm SD (n = 3). Different superscript letters (a–d) indicate statistically significant differences between groups according to the Games–Howell post hoc test ($p < 0.05$). Treatments sharing the same letter are not significantly different.

One-way ANOVA demonstrated a significant overall effect of treatment on inhibition zone diameter ($p < 0.05$). Post hoc analysis using the Games–Howell test, reflected by the superscript letters in Table 2, showed that the 50%:50% and 75%:25% combinations (groups ^b and ^c) produced significantly larger inhibition zones than the 25%:75% combination and the negative control (group ^a), indicating a clear improvement

in antibacterial activity at higher red onion proportions. The positive control formed a separate group (^d) with the greatest inhibition, significantly different from all extract combinations. The 25%:75% combination shared the same superscript letter as the negative control (^a), indicating no significant difference between them. Taken together, these findings suggest that the antibacterial effect of the 25%:75% combination is minimal, whereas the 50%:50% and particularly the 75%:25% combinations provide more meaningful inhibitory activity against *S. aureus*, although still markedly weaker than ciprofloxacin.

Comparison with previous studies

The inhibition zones obtained in this study are in line with previous work on red onion and ginger extracts. Widhorini and Rafianti reported that red onion extract alone inhibited *S. aureus* with a mean inhibition zone of approximately 14 mm at a 5% concentration [15]. Azkiyah found that ginger rhizome extract produced inhibition zones of 8.17–12.33 mm against *S. aureus* at concentrations of 5–80% [10]. Harlita et al. reported inhibition zones of 8.00–9.89 mm for combinations of dayak onion and red ginger extracts against *Salmonella typhi* [8], while Pohan et al. observed an inhibition zone of 11.4 mm for red ginger and kencur extract combinations at 100% red ginger concentration against *S. aureus* [9].

Compared with these studies, the inhibition zones observed for the red onion–white ginger combinations in the present work (maximum 7.80 mm) are smaller. Several factors may account for these differences, including variations in plant variety and origin, extraction methods, solvent systems, concentrations tested, and differences in the bacterial strains or inoculum size. In addition, crude extracts contain a complex mixture of active and inactive constituents, and possible antagonistic interactions between some components may reduce the overall antibacterial effect at certain combination ratios. The finding that the 75%:25% combination was more active than the 25%:75% combination suggests that red onion constituents may contribute more strongly to the observed inhibition than white ginger constituents at the tested concentrations.

Although the combined extracts demonstrated clear antibacterial activity, their effect remained markedly weaker than ciprofloxacin, which produced an inhibition zone almost four times larger than the most active combination. This indicates that, in their current crude form and at the tested concentrations, the red onion–white ginger combinations cannot be considered substitutes for standard antibiotic therapy. However, they may have potential as complementary agents or as a basis for the development of topical preparations or functional herbal products aimed at supporting infection control.

Limitations and implications

This study has several limitations. First, only one *S. aureus* strain was tested, so the results may not represent the full range of susceptibility among clinical isolates with diverse resistance profiles. Second, the antibacterial activity was assessed solely by the agar well diffusion method, without determination of minimum inhibitory concentration (MIC) or time–kill kinetics, which would provide more detailed information on the potency and bactericidal or bacteriostatic nature of the extracts. Third, the extracts used were crude ethanol extracts without standardisation of specific active constituents, making it difficult to attribute the observed effects to particular compounds or to compare doses across studies.

Future research should include MIC and time–kill assays, evaluate activity against a broader panel of clinical *S. aureus* isolates, and isolate or enrich the principal

active components from the extracts. In-vivo studies and formulation development, particularly for topical use, are also needed to explore the potential role of red onion and white ginger combinations as adjunctive antibacterial agents.

4. Conclusion

This study showed that combined ethanol extracts of red onion and white ginger are capable of inhibiting the growth of *Staphylococcus aureus* in vitro. The magnitude of the antibacterial effect varied according to the proportion of each extract, with the 75%:25% red onion:white ginger combination producing the largest inhibition zone, followed by the 50%:50% combination, while the 25%:75% combination demonstrated only minimal activity and was comparable to the negative control. All extract combinations were substantially less potent than ciprofloxacin, which produced the greatest inhibition. These findings indicate that red onion-white ginger extract combinations possess modest antibacterial activity against *S. aureus* and may serve as a preliminary basis for the exploration of plant-based antibacterial adjuncts.

Acknowledgements:

The authors gratefully acknowledge the support of the Abdurrab University for all their support in this research and all those who have helped in this research.

Conflicts of Interest:

The author declares that there are no conflicts of interest in this research.

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