

## RESEARCH ARTICLE

**Antimicrobial Activity of Green Tea: Honey Blend against  
Acne-Causing Microorganism**

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**ABSTRACT**

Acne, a prevalent dermatological condition affecting millions globally, is predominantly attributed to the colonization and proliferation of specific microorganisms on the skin. This microbial overgrowth triggers inflammation, comedone formation, and ultimately leads to the manifestation of acne lesions. In recent years, increasing attention has been directed toward natural alternatives for acne management, particularly the use of green tea and honey, owing to their well-documented antimicrobial properties. In this study, acne-associated microorganisms were isolated and subjected to biochemical and physiological analyses for confirmation. The antimicrobial efficacy of a green tea–honey formulation was evaluated against selected acne-related pathogens, including *Candida* spp., *Staphylococcus* spp., and *Pseudomonas* spp., using zone of inhibition assays. The results demonstrated that the green tea–honey blend exhibited significant antimicrobial activity, with notable inhibition zones observed against all tested microorganisms. These findings indicate that the green tea–honey combination possesses substantial potential as a topical antimicrobial agent. Accordingly, a spot treatment formulation incorporating this blend was developed for prospective application in acne therapy.

**Keywords:** Antimicrobial, *Candida* spp., green tea-honey blend, *Pseudomonas* spp., *Staphylococcus* spp.

**INTRODUCTION**

Green tea (*Camellia sinensis*) has long been recognized for its health-promoting properties, largely attributed to its high content of antioxidants, particularly catechins such as epigallocatechin gallate (EGCG), epigallocatechin, and epicatechin gallate. These polyphenolic compounds exhibit notable antimicrobial activity against a range of pathogens and have shown promise in treating skin conditions like acne.<sup>[1]</sup> Green tea also provides anti-inflammatory and sebum-regulating effects, which are beneficial in reducing acne severity and promoting skin healing. Similarly, honey has been used for centuries in traditional medicine due to

its natural antibacterial, antifungal, and wound-healing capabilities. Its efficacy is attributed to factors such as hydrogen peroxide content, high viscosity, acidic pH, and natural enzymes that inhibit microbial growth while maintaining skin moisture and supporting tissue regeneration.<sup>[2]</sup>

Acne is a common chronic inflammatory skin condition that develops due to multiple factors, including hormonal fluctuations, increased sebum production, clogged pores, and microbial colonization. Key microbial agents implicated in acne pathogenesis include *Propionibacterium acnes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, all of which contribute to inflammation and lesion development.<sup>[3]</sup> Recent studies suggest that combining green tea and honey may offer synergistic effects in acne management by inhibiting these microorganisms, reducing inflammation, and enhancing skin repair. Green

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tea's antioxidant and antimicrobial catechins, combined with honey's antibacterial and wound-healing properties, present a promising natural treatment for acne. This study aims to evaluate the antimicrobial activity of a green tea-honey blend against acne-associated microorganisms and explore its potential integration into skincare formulations.

## MATERIALS AND METHODS

Pus specimens were aseptically collected from inflamed acne lesions of 10 patients (3 males, 7 females). After cleansing the affected area with saline, pus was extracted using sterile cotton swabs and transferred into leak-proof containers. Samples were streaked on blood agar and Mannitol Salt Agar and incubated at 37°C for 24 h. Three distinct isolates were obtained using the streak plate method for pure colony isolation.<sup>[4]</sup>

### Morphological and Biochemical Characterization

Isolates were characterized through Gram staining, which differentiated Gram-positive and Gram-negative bacteria.<sup>[5]</sup> Germ tube tests were used to identify *Candida* spp.<sup>[6]</sup> Biochemical tests, including indole, methyl red, voges-proskauer, citrate utilization, catalase, oxidase, urease, and thyroid-stimulating immunoglobulin tests, were conducted according to standard microbiological procedures.<sup>[5,7]</sup>

### Preparation of Green Tea and Honey Extracts

Green tea extract was prepared by mixing 15 g of dried green tea powder with 100 mL of sterile distilled water, followed by filtration and refrigeration.<sup>[8]</sup> Honey extract was prepared by diluting raw honey to the required concentration and incubating at 37°C for 30 min.<sup>[9]</sup>

### Preparation of Green Tea-Honey Blend

Equal volumes (5 mL each) of green tea extract and honey were combined to prepare the blend

used in subsequent testing.<sup>[10]</sup> Green tea-honey extract was subjected to gas chromatography-mass spectrometry (GC-MS) analysis to identify active bio-compounds. Standard procedures were followed for sample injection, calibration, and compound identification using retention times and mass spectra.<sup>[11]</sup>

### Antimicrobial Activity Assessment

The antimicrobial activity of green tea, honey, and their combination (TS1, TS2, TS3) was evaluated using the Kirby-Bauer disc diffusion method. Inoculum was prepared by suspending bacterial colonies in saline and adjusting turbidity to the 0.5 McFarland standard.<sup>[12]</sup> Plates were incubated at 37°C for 24 h, and zones of inhibition were measured.

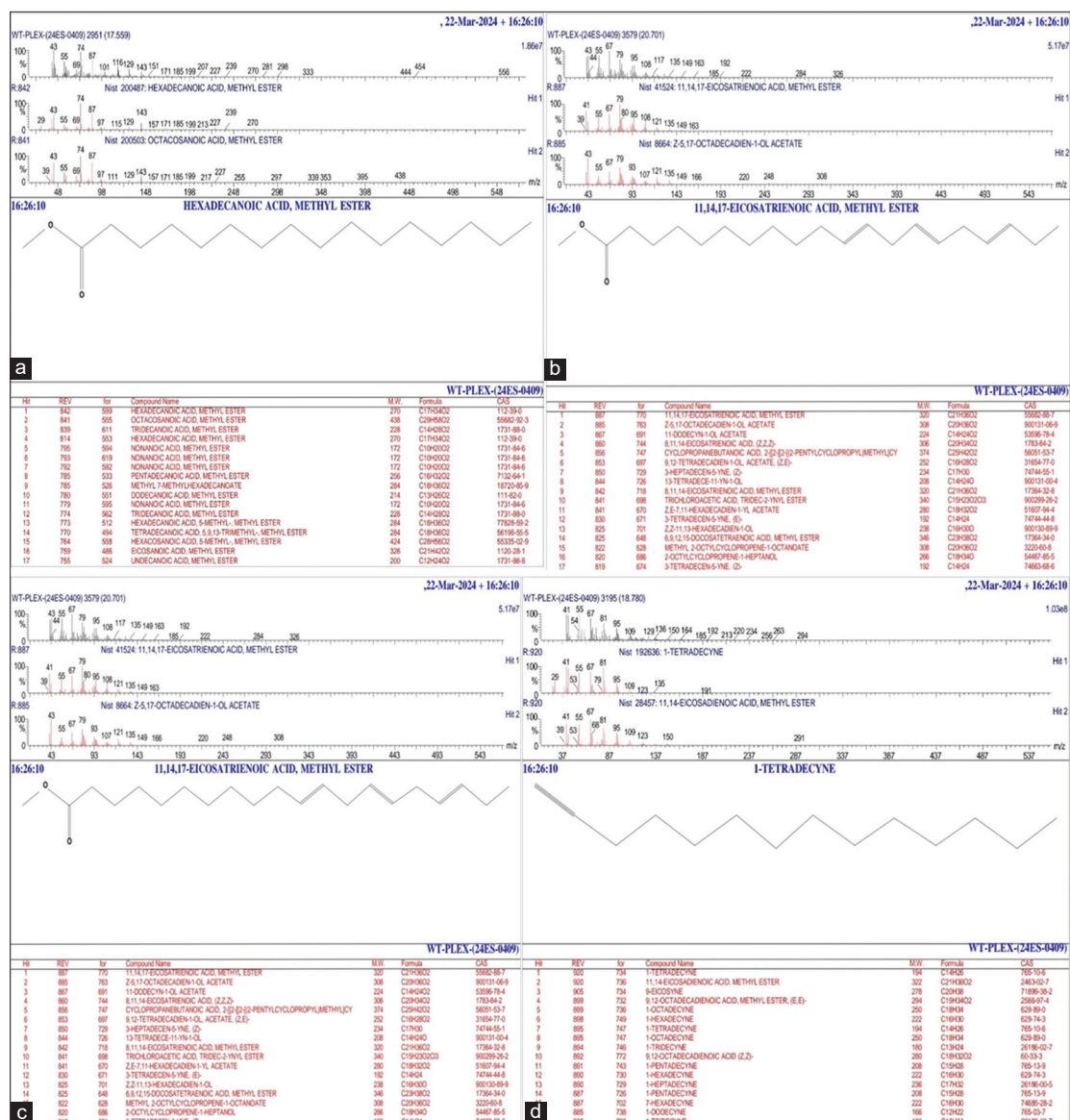
Disc diffusion tests were also performed against fungal isolates on glucose-supplemented Mueller-Hinton agar with methylene blue dye. Antifungal zones of inhibition were recorded post-incubation.<sup>[13]</sup>

### Acne Spot Treatment Cream Preparation

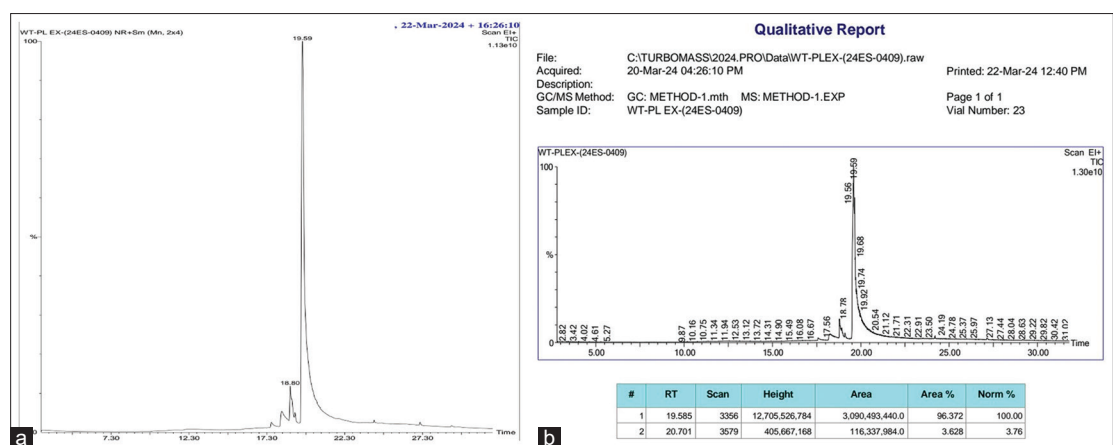
A topical cream was formulated by melting beeswax and blending it with green tea extract and raw honey. Optional essential oils were added, and the mixture was cooled and stored in sterile glass jars.

## RESULTS

All the selected isolates (S1, S2, and S3) were identified on the basis of colony/cell morphology and biochemical test as *Candida* spp., *Pseudomonas* spp. and *Staphylococcus* spp., respectively. 100 mL each green tea, honey, and (Green tea and honey) blend was prepared and analyzed using GC-MS. Six compounds were found in the analysis [Figures 1 and 2]. Further antimicrobial studies showed that green tea and honey blend showed the maximum antimicrobial activity against the acne-causing pathogens compared to green tea and honey alone [Tables 1-3 and Figure 3]. Acne spot treatment cream was prepared using the green tea and honey blend for future prospects [Figure 4].

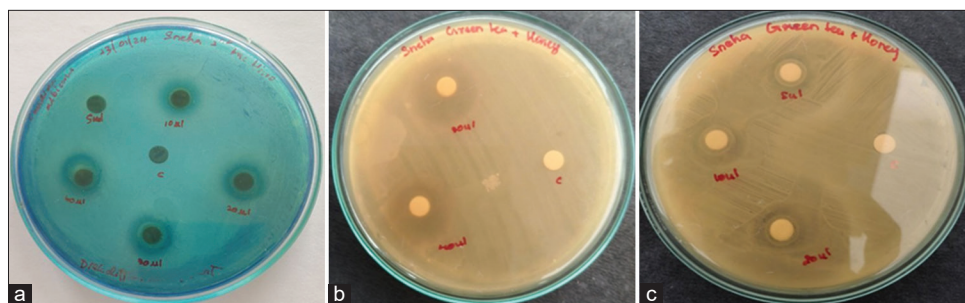


**Figure 1: (a and b) Gas chromatography-mass spectrometry result of green tea and honey blend. (a) hexadecanoic acid, methyl ester (b) 9,12-Octadecadienoic acid (z,z) (c) 11,14,17- eicosatrienoic acid (d) 1- tetradecyne methyl ester**



**Figure 2: (a and b) Chromatography report**





**Figure 3:** Antimicrobial activity of green tea+honey blend against acne causing microorganisms (a) *Candida* spp. (b) *Pseudomonas* spp. (c) *Staphylococcus* spp.



**Figure 4:** Acne spot treatment cream

## DISCUSSION

Acne vulgaris is a multifactorial inflammatory skin disorder influenced by hormonal imbalance, excess sebum production, follicular hyperkeratinization, and microbial colonization. Among the microorganisms involved, species such as *Staphylococcus*, *Pseudomonas*, and *Candida* play a significant role in triggering inflammation and lesion development. In light of rising antibiotic resistance, natural remedies such as green tea and honey have gained attention for their antimicrobial and anti-inflammatory properties. Green tea, derived from *C. sinensis*, contains EGCG, a polyphenol known to inhibit bacterial growth by disrupting cell membranes, preventing adhesion, and downregulating pro-inflammatory cytokines.<sup>[14]</sup> Similarly, honey exerts broad-spectrum antimicrobial activity attributed to its low pH, high osmolarity, and bioactive compounds including hydrogen peroxide and methylglyoxal.<sup>[15]</sup> This study demonstrated that both green tea and honey individually possess antimicrobial activity,

**Table 1:** Antibacterial activity test result against *Staphylococcus* spp.

| Concentrations ( $\mu$ L) | Green tea | Honey | Green tea+honey |
|---------------------------|-----------|-------|-----------------|
| 5 $\mu$ L                 | 10 mm     | 14 mm | 19 mm           |
| 10 $\mu$ L                | 14 mm     | 15 mm | 20 mm           |
| 20 $\mu$ L                | 16 mm     | 19 mm | 25 mm           |
| 30 $\mu$ L                | 17 mm     | 20 mm | 30 mm           |
| 40 $\mu$ L                | 19 mm     | 21 mm | 35 mm           |

**Table 2:** Antibacterial activity test result against *Pseudomonas* spp.

| Concentrations ( $\mu$ L) | Green tea | Honey | Green tea+honey |
|---------------------------|-----------|-------|-----------------|
| 5 $\mu$ L                 | 10 mm     | 14 mm | 19 mm           |
| 10 $\mu$ L                | 14 mm     | 15 mm | 20 mm           |
| 20 $\mu$ L                | 16 mm     | 19 mm | 25 mm           |
| 30 $\mu$ L                | 17 mm     | 20 mm | 30 mm           |
| 40 $\mu$ L                | 19 mm     | 21 mm | 35 mm           |

**Table 3:** Antibacterial activity test result against *Candida* spp.

| Concentrations ( $\mu$ L) | Green tea | Honey | Green tea+honey |
|---------------------------|-----------|-------|-----------------|
| 5 $\mu$ L                 | 10 mm     | 7 mm  | 15 mm           |
| 10 $\mu$ L                | 13 mm     | 10 mm | 20 mm           |
| 20 $\mu$ L                | 14 mm     | 11 mm | 21 mm           |
| 30 $\mu$ L                | 14 mm     | 12 mm | 25 mm           |
| 40 $\mu$ L                | 15 mm     | 12 mm | 26 mm           |

but their combination showed enhanced efficacy. The green tea-honey blend produced the largest zones of inhibition against *Candida* spp., *Pseudomonas* spp., and *Staphylococcus* spp., suggesting a synergistic effect that targets multiple microbial mechanisms. These findings align with previous research highlighting the complementary actions of plant-based and natural antimicrobial agents. Moreover, a topical acne cream formulated

using this blend could provide a safe, natural alternative to conventional treatments. Its ability to reduce microbial load while soothing inflammation may aid in preventing scarring and promoting skin healing. Thus, the green tea-honey combination presents a promising adjunctive strategy in acne management, especially in cases of antibiotic resistance or sensitivity to synthetic products.

## CONCLUSION

The findings of this study highlight the promising antimicrobial potential of a green tea–honey blend against key acne-associated microorganisms, including *Candida* spp., *Staphylococcus* spp., and *Pseudomonas* spp. The significant zones of inhibition observed affirm the blend's ability to combat microbial overgrowth that contributes to acne pathogenesis. These results support the use of natural ingredients, such as green tea and honey, as effective and safer alternatives to conventional antimicrobial agents. Furthermore, the development of a spot treatment formulation based on this blend offers a practical and accessible approach for managing acne. Future studies may focus on clinical evaluations and optimization of the formulation to enhance its therapeutic efficacy and stability.

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