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RESEARCH ARTICLE

Antimicrobial Activity of Green tea and Mint Extract against Wound Pathogens

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ABSTRACT

Introduction: Wound healing may also be affected by the growth of certain wound pathogens including Pseudomonas, Escherichia coli, Staphylococcus aureus. Camellia sinensis, or the Green tea, has large quantities of catechins and their derivatives called polyphenols which are known for their antioxidant, anti-cancer effects. Mint or Mentha is a plant, also having established antimicrobial, antioxidant, anticancer, antiemetic, and blood purification properties.

Aim: To establish the antimicrobial properties of Green Tea and Mint extract on wound pathogens

Materials and Methods: The plant extract was prepared with 1g dried green tea leaves and 1g dried mint leaves boiled for 10 minutes in 100ml distilled water, at 60-80°C on a heating mantle. The extract was filtered using Whatman No.1 filter paper. Three Mueller-Hinton agar plates with microbial suspensions of Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus dispensed on each of their four wells, were prepared. Three of the four wells in a plate were suspended with 25 µL, 50 µL, 100 µL of the prepared extract, with the fourth as a standard. The prepared plates were then incubated for 24 hours at 37°C, and the zone of inhibition for each sample was recorded.

Results: At 100 µL of the extract for all the three organisms, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus, 25mm, 28mm and 27mm were the zones of inhibition obtained, which were the maximum recorded.

Conclusion: In the present study, it is observed that the higher the concentration of the extract, greater is the zone of inhibition for the extract against the organism tested.

KEYWORDS:
Camellia sinensis, Mentha, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, antimicrobial activity, wound pathogens

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INTRODUCTION

A wound can present as a disruption in the epithelial structures of the skin, or even a tissue, muscle or other structures of the body (1). The prime concern regarding wounds is facilitating their healing. There are several factors that disrupt healing, including unnecessary movement, lack of blood supply, vitamin deficiency, clotting or collagen disorders, and mainly, superadded infections (2). Infections may slow down the process of healing or even cause scars and spread onto adjacent structures (3). Common wound pathogens include Pseudomonas, Escherichia coli, Staphylococcus aureus (4), and Enterococci (5). Subcutaneous tissues, on exposure due to a wound, provides an environment favourable to the growth of these pathogens, allowing their colonization at the site of the wound (6). Wound healing may also be affected by factors

pertaining to the wound including the type of the wound, the depth, its site, and even the immunity of the host to any invading pathogens (7). Hence, until a fully recovered intact epidermis, or any other structures, pertaining to the injury, are formed, it is necessary to keep the wound free of any foreign organism invasion (8).

In several Asian and African countries, several medicinal plants (9–12) are used as drugs, especially for treating infectious diseases that are resistant to a wide range of allopathic medicines (13). They are also known for their cost-effectiveness and their wide spectrum of targeted organisms (14). One such plant harnessed for its antimicrobial benefits is the Green tea. Camellia sinensis, or the Green tea, has large quantities of catechins and their derivatives called polyphenols (15) which are known for their antioxidant , anti-cancer effects as well as for their beneficial effects on the CVS (16). Previous literature has shown that the moderate inclusion of green tea in one's daily diet prevents susceptibility to a wide range of bacteria (17) including Staphylococcus aureus, Vibrio cholerae, Clostridium perfringens and several other gram negative bacteria (18) The present study thus aims to assess the antimicrobial effect of green tea extracts, on three organisms, Pseudomonas, S. aureus and E. coli, which are wound pathogens.

Mint or Mentha is a plant with established pharmacological properties including antimicrobial, antioxidant (19), anticancer, antiemetic, and blood purification properties (20). The biologically active compound in mint, Pulegone, attributes to the medicinal properties of the plant (21). Several other bioactive compounds in mint are menthol, borneol, menthone (22), isomenthone, and piperitenone (23). Previous literature shows essential oils derived from mint show a significant antimicrobial property against E. coli, S. typhimurium, A. flavus, A. niger and P. aeruginosa (20). However, the combined antimicrobial effect of Green tea and mint extracts have not been assessed earlier. Our team has extensive knowledge and research experience that has been translated into high quality publications (24-28),(29),(30),(31),(32),(33),(34),((26,35,36),(37–41),(42),(43),(44).

MATERIALS AND METHODS

The study used bacterial cultures of Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus which were isolated and collected from the Saveetha Dental College and Hospital. The plant extract was prepared with 1g dried green tea leaves and 1g dried mint (Fig. 1) leaves boiled for 10 minutes in 100ml distilled water, at 60-80°C on a heating mantle (Fig. 2). The extract was filtered using Whatman No.1 filter paper (Fig. 3). The filtrate was further boiled again for 10 minutes at 60-80°C and filtered again. The extract thus prepared was used for the study. The agar well diffusion method was used to assess the antimicrobial activity of the extract prepared. Three Mueller-Hinton agar plates with microbial suspensions of Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus dispensed on each of their four wells, were prepared. Three of the four wells in a plate were suspended with different concentrations of the prepared extract, being 25 µL, 50 µL, 100 µL of the prepared extract, with the fourth as a standard. The prepared plates were then incubated for 24 hours at 37°C. Following this, the zone of inhibition for each sample was recorded, and the findings were tabulated.

RESULTS

In the present study, the tests for the antimicrobial activity of the Green tea and Mint extracts were done using the agar well diffusion method. Three agar plates were used for assessing the inhibitory effect of the extract on Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. Each plate had 4 wells each with different nanoparticle concentrations being 25 µL, 50 µL, 100 µL while the fourth was a standard. Against Pseudomonas aeruginosa, the diameter of the zone of inhibition at 25 µL, 50 µL, 100 µL and for the antibiotic was observed to be 17 mm, 20 mm, 25 mm, and 17mm respectively (Fig. 4). With Escherichia coli, the diameter of the zone of inhibition of the extract at 25 µL, 50 µL, 100 µL and the antibiotic was obtained as 17 mm, 25 mm, 28 mm and 18 mm, respectively (Fig. 5). Against Staphylococcus aureus, the diameter of the zone of inhibition at 25 µL, 50 µL, 100 µL and the antibiotic was observed as 18 mm, 25 mm, 27 mm and 17 mm, respectively (Fig. 6). Thus, maximum activity for all the three was observed at 100 µL (Graph 1).

DISCUSSION

It is inferred from the present study that green tea and mint extract shows an evident antimicrobial activity against wound pathogens. The combined antimicrobial effect of both the components were thus assessed. Previous findings (45) have individually assessed the compounds. Huber et al (46) has discussed the polyphenolic compounds in tea extracts to be intervening with the metabolic pathways of bacteria (47). Hoshino et al (48) has assessed the activity of catechins bound to copper complexes on E. coli bacteria. Shalayel et al assessed the antimicrobial properties of ethyl extracts of mint (49) against S. aureus bacteria. In their study, Mancuso et al (50) described the activity of mint extracts against P. aeruginosa. (40) (51) (52) (53) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) (64). In the present study, it is observed that the higher the concentration of the extract, greater is the zone of inhibition for the extract against the organism tested. Likewise, at 100 µL of the extract for all the three organisms, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus, 25mm, 28mm and 27mm were the zones of inhibition obtained, which were the maximum recorded. These values were higher than even the values recorded for the antibiotic, indicating the extract is a more potent antimicrobial agent than the antibiotic, and is a possible drug candidate.

CONCLUSION

From the present study, it can be concluded that there is a considerable antimicrobial activity displayed by green tea and

mint extract at high concentrations. The extract is thus a potential drug candidate which can be employed as a less biotoxic alternative to existing chemically synthesized formulations.

Fig.1: Mixture of 1mg each of dried Green tea and Mint leaves

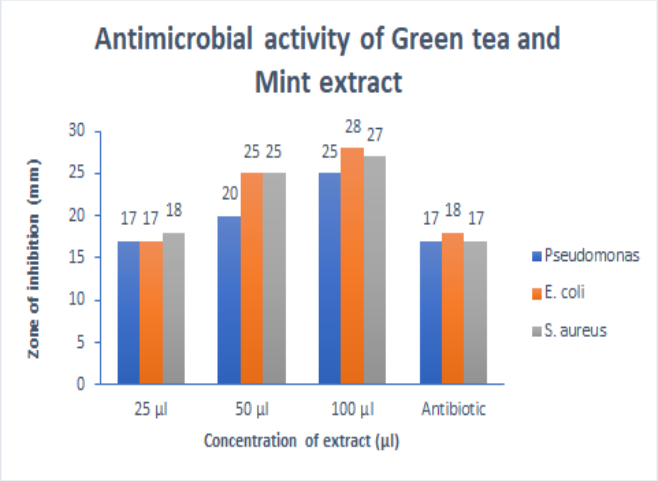
Fig.2: The extract is boiled on the heating mantle

Fig.3: Filtering of extract prepared using Whatman no. 1 filter paper

Fig.4: P. aeruginosa culture plate tested for the Green tea and mint extract's antimicrobial activity using disc-diffusion method.

Fig.5: E. coli culture plate tested for the Green tea and mint extract's antimicrobial activity using disc-diffusion method.

Fig.6: S. aureus culture plate tested for the Green tea and mint extract's antimicrobial activity using disc-diffusion method.



Graph 1: Graph representing the zone of inhibition obtained for each sample for different concentrations of the extract. The X-axis denotes the concentration in µL while the Y-axis shows the zone of inhibition in mm. Blue represents P. aeruginosa, orange represents E. coli, grey represents S. aureus. It is evident from the graph that maximum inhibitory activity was observed with a concentration of 100 µL for each.

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