ORIGINAL ARTICLE

Anti-inflammatory and antimicrobial effects of herbal formulation using karpooravalli, mint, and cinnamon on wound pathogens

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ABSTRACT

Wound damage must be shielded from inflammatory responses caused by the external environment, while also receiving adequate moisture and promoting wound healing. *Cinnamomum verum*, *Coleus aromaticus/amboinicus*, and *Mentha* have proven effects on anti-inflammatory and antimicrobial effects as separate in various studies, but no studies have observed the synergistic effects of one herb on the other. The aim of the present study is to assess the anti-inflammatory and antimicrobial properties of karpooravalli, mint, and cinnamon formulation against wound pathogens for better healing of mouth ulcers through an *in vitro* study for further mouth paint preparation. Herbal extract is prepared using herbal plants karpooravalli, mint, and cinnamon. We studied the antimicrobial effects on *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* with ampicillin antibiotic disks as positive control and anti-inflammatory with control of diclofenac sodium. The antimicrobial activity of zone of inhibition increased with higher concentration of 100 µL against *E. coli*, *S. aureus*, and *Pseudomonas* at 20 mm, 18 mm, and 15 mm, respectively. The anti-inflammatory activity showed the highest percentage of inhibition using 50 µL with 91.5%; this reveals good anti-inflammatory activity by the synergistic action of the herbal formulation. The synergistic effect of the herbal formulation had a comparable anti-inflammatory activity as that of the standard. Hence, it can be employed in large-scale production and may be used for fabricating a natural product based on mouthwashes and mouth paints.

Key words: Antimicrobial, antioxidant, cinnamon, karpooravalli, mint

INTRODUCTION

Inflammation is a protective mechanism that propagates the inflammatory response by a sequence of various biochemical events involving the local vascular system, the immune system, and numerous cells in the wound tissue.[1] As a result, wound damage must be shielded from inflammatory responses brought on by the external environment, while also maintaining adequate moisture

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and aiding wound healing.[2] Antimicrobial wound dressings can help reduce the risk of bacterial infection in the healing process of wounds.[3]

Cinnamomum verum (cinnamon) is a member of the *Lauraceae* family, which has long been employed in traditional medicine. Cinnamon barks have been used as a spice, condiment, and for flavoring.[4] Some of its properties include antioxidant, antibacterial, antidiabetic, hypoglycemic, hypolipidemic, and anti-inflammatory properties. The anti-inflammatory effects of cinnamon components such as cinnamaldehyde, 2-hydroxycinnamaldehyde, and quercetin have already been proven by previous literature.[5-7] Cinnamon alcoholic and aqueous extracts have also been shown to speed wound healing due to their antioxidant qualities reported in a few studies.[8]

Coleus aromaticus/amboinicus is a big succulent aromatic perennial herb belonging to the *Lamiaceae (Labiatae)* botanical family and genus *Coleus* (currently referred to as *Plectranthus*). Indian borage, country borage, French/Spanish thyme, Indian mint, Mexican mint, Cuban oregano, soup mint, and karpooravalli are various English names for this plant, and this has been used commonly to treat chronic asthma, bronchitis, malaria, hepatitis, renal/vesical calculi, convulsions, and epilepsy in traditional medicine. Furthermore, karpooravalli leaf juice mixed with honey is good for colds and coughs, and the heated concentrated decoction is good for respiratory infections.[9] *Plectranthus amboinicus* active ingredients have been demonstrated to inhibit AP-1 and TNF and increase wound contraction, resulting in wound healing, increasing deposition of collagen, and shortening epithelialization of the wound.[10,11]

Mentha is a genus of plants from the *Lamiaceae* family and is generally known as mint,[12] which is an aromatic perennial herb.[13] According to modern pharmacology studies, the complete herb of *Mentha piperita* includes antioxidant, antiviral, cytotoxic, antibacterial, and antiallergenic properties.[14,15] The common pathogens in the oral cavity such as *E. coli*, *Pseudomonas*, and *S. aureus* might reduce wound healing. Mouth ulcer wound healing has to be taken care of to reduce the inflammation resulting in further healing, along with antimicrobial effect against common oral pathogens. Nobody has performed any research to evaluate the efficacy of the antimicrobial and anti-inflammatory properties of a combination of karpooravalli, mint, and cinnamon herbal formulation. The study's main rationale is to evaluate the antibacterial and antioxidant capabilities of karpooravalli, mint, and cinnamon formulation against wound pathogens for better healing of mouth ulcers through an *in vitro* study for future mouth paint preparation.

MATERIALS AND METHODS

Preparation of herbal extract

One gram each of the herbal plants was taken in 100 mL of distilled water such as karpooravalli, mint, and cinnamon, which were dissolved and stored in a beaker overnight, as depicted in Figure 1. Initially, the water was boiled for 15 min before filtered using Whatman filter paper. The preparation was further boiled for 30–60 min to yield a concentrated extract and then was stored at a low temperature below 20°C for later use [Figure 2].

Antimicrobial

Fresh bacterial cultures were made on Hi-Veg broth medium, where 10 µL cultures of *E. coli*, *S. aureus*, and *Pseudomonas* were inoculated. These plates were incubated in a shaker for 18 h. A nutrient agar medium was prepared and 5 mm wells were made and different concentrations (25–75 µg/ml) of extract from Karpuravalli, Mint and Cinnamon were added, along with one positive control with antibiotic ampicillin disk [Figure 3]. Before measuring zones of inhibition (ZOI), these plates were incubated at 37°C for 18 h.[16]

Anti-inflammatory

1% bovine albumin fraction of 2 mL and 400 L of plant extract were blended in varying concentrations (500–100 µg/mL). The response mixture was altered to maintain the pH at 6.8 using 1N HCl. The response mixture was incubated at room temperature for 20 min in a water bath. Before measuring the absorbance at 660 nm, the mixture was allowed to cool to room temperature. An equivalent quantity of plant extract was replaced with control DMSO, and diclofenac sodium served as the standard. The experiment was performed in triplicate % Inhibition was calculated using the following formula:

Percentage of inhibition = Control OD − Sample OD divided by Control OD.[17]

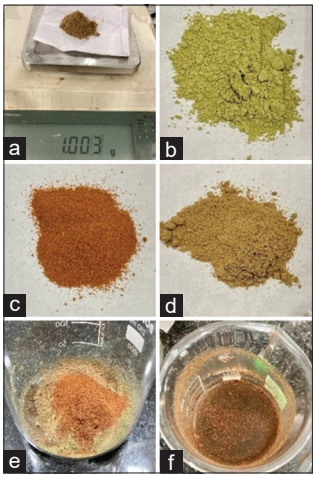

Figure 1: Equal measurement of 1 g (a) of mint (b), cinnomon (c), and karpooravalli (d) is dissolved in distilled water (e and f)

RESULTS AND DISCUSSION

Antimicrobial activity

ZOI of the extraction against bacterial culture was demonstrated, as depicted in Figure 4.

ZOI increased with higher concentration of 100 µL toward *E. coli*, *S. aureus*, and *Pseudomonas;* with 20 mm, 18 mm, and 15 mm, respectively, among which the highest antimicrobial activity was found against *E. coli*. Antimicrobial activity showed good results in comparison to control. The least results were obtained with lower concentration of extract (25 µL) with 9 each, which is summarized in Table 1.

Cinnamon oil is effective against various strains; a study by Atki *et al*. in 2019 showed effective results toward *Staphylococcus*, *E. coli*, and *Pseudomonas aeruginosa* similar to the present study.[18,19] The major action of trans-cinnamaldehyde is important for the highest antibacterial effect.[20] *C. amboinicus* oil (karpooravalli) is effective against bacteria and fungi; ethanolic extract reported greater antibacterial activity of karpooravalli with higher concentration (1000 µL) against *Staphylococcus*, *E. coli*, and *P. aeruginosa* by Ramalakshmi *et al*. in 2014, similar to the present study with less concentration of 100 µL. Furthermore, it is effective against other species such as *Salmonella typhi*, *Klebsiella pneumoniae*, and *Proteus* species.[21]Another study found that the inhibitory effect was greater in Gram-positive than Gram-negative bacteria.[22]*Mentha piperita* oil inhibited *S. aureus*, *Staphylococcus pyogenes*, and *K. pneumonia* more effectively and lesser against *E. coli*; more effective results against *S. aureus* (Gram-positive) than that of other species were not in concordance with the present study.[23] This is because Gram-negative bacteria possess thicker liposaccharide cell walls than Gram-positive bacteria, leading to reduced permeability.[24] *Mentha* is also effective against other species such as *Staphylococcus epidermidis*, *Candida albicans*, *Vibrio cholerae*, and *Bacillus cereus* observed by researches with ZOI 13–21 mm.[25]

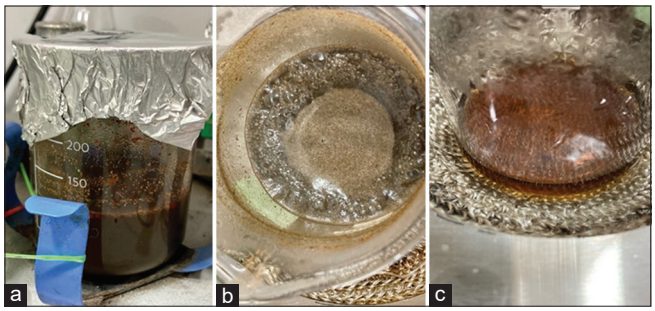

Figure 2: Preparation of concentrated extract (herbal formulation). (a) The mix was stored overnight, (b) Boiling of filtered mixture to make the solution concentrated and (c) Concentrated herbal extract

Our study showed that ZOI of the herbal extract is effective against *E. coli*, a more sensitive Gram-negative organism than that of *Pseudomonas* and Gram-positive organism *S. aureus*; this may be due to synergistic effect of the extract. The goal of herbal formulation in folk medicine is to achieve synergistic interactions between different plant extracts.[18]

Anti-inflammatory activity

In present study, the anti-inflammatory effect of herbal formulation prepared from Karpooravalli, Mint and Cinnamon was studied with different concentrations like 10 µL, 20 µL, 30 µL, 40 µL and 50 µL. 50 µL was the highest concentration showing more percentage of inhibition of 91.5%, which demonstrates good anti-inflammatory properties due to the synergistic action of the herbal formulation [Figure 5].

Previous literature has found that different pharmacological plants have anti-inflammatory properties also, karpooravalli, cinnamon, and mint separately. Recent studies associated with cinnamon show anti-inflammatory effects in

Table 1: Antibacterial activity of plant extract containing karpooravalli, mint, and cinnamon

Concentration of the extract	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas*
25 µL	9	9	9
50 µL	17	13	9
100 µL	20	18	15
Ampicillin	31	21	20

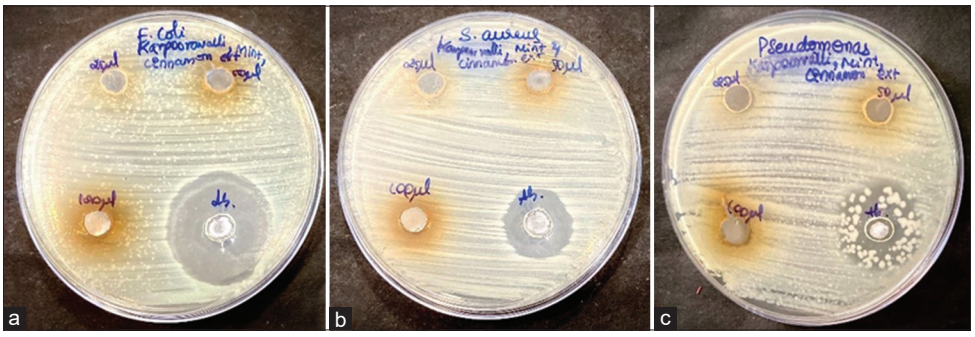

Figure 3: Nutrient agar medium showing antimicrobial effect of karpooravalli, mint, and cinnamon. (a) *E. coli*: *Escherichia coli*; (b) *S. aureus*: *Staphylococcus aureus* and (c) Pseudomonas

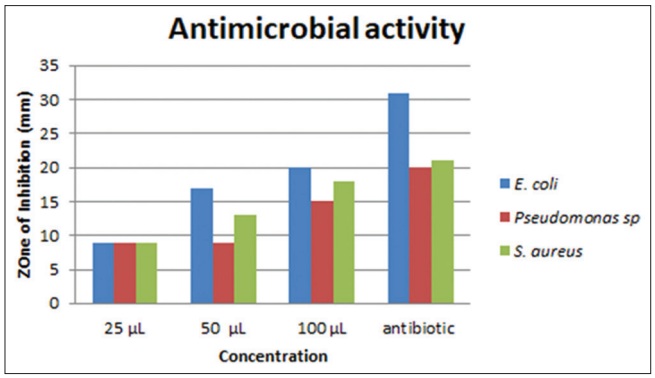

Figure 4: Graph represents the zone of inhibition of herbal formulation against the pathogens such as *E. coli*, *Pseudomonas*, and *S. aureus* inoculation in 5 mm wells. *E. coli*: *Escherichia coli*; *S. aureus*: *Staphylococcus aureus*

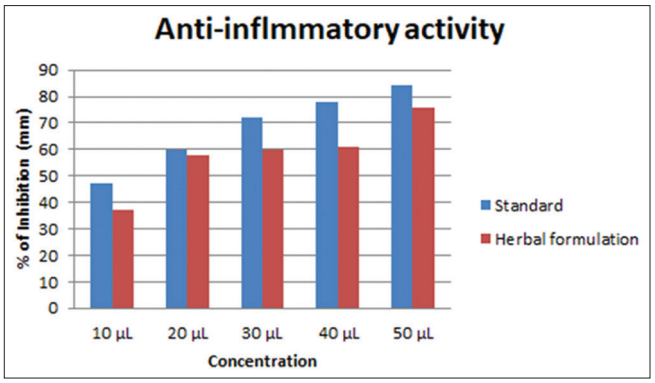

Figure 5: The graphical representation of herbal formulation mediated anti-inflammatory effect. X-axis shows concentration used (µL) and Y-axis shows percentage inhibition. Maximum inhibitory effect shown at 50 µL

the nervous system by suppressing the expression of cyclooxygenase-2, inducible nitric oxide synthesis, and nitric oxide production.[19] *P. amboinicus* has significantly reduced malondialdehyde, TNF-, and COX-2 levels ($P < 0.001$).[26] A previous study by Sun *et al.*, 2014 has shown that the anti-inflammatory characteristics of the essential oil extracted from Chinese-grown *Mentha piperita* leaves have already been reported to reduce edema in a dose-dependent way with 30.24% at 800 µg.[15] Another study has shown that the methanolic extract with combination of *Adhatodavasica nees* and *Mentha piperita* shows better anti-inflammatory activity as a synergistic effect than that of the control diclofenac sodium.[27] The present study shows the synergistic activity of herbal formulation using commonly available herbs with lesser side effects. Further studies can be done for cytotoxicity of the herbal formulation.

CONCLUSION

Standard synthetic drugs have proven anti-inflammatory properties; however, still, various side effects are reported; hence, the herbal formulation prepared from karpooravalli, mint, and cinnamon in the present study is a natural source. Our current research reveals that *Pseudomonas* species, *S. aureus*, and *E. coli* had a larger ZOI at 100 µg/mL. Synergistic effect of the herbal formulation had a comparable anti-inflammatory activity to that of the standard. Hence, it can be employed in large-scale production and may be used for fabricating a natural product based on mouthwashes and mouth paints.

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Conflicts of interest

There are no conflicts of interest.

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