Evaluation of Anti-microbial activity of *Aloe vera, Chamomile and Propolis* formulation against Oral Pathogens

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Abstract:

Background: The increasing prevalence of antibiotic resistance and the side effects of synthetic oral care products have prompted the exploration of natural alternatives for managing oral infections. Aloe Vera, chamomile, and propolis are known for their individual antimicrobial, anti-inflammatory, and healing properties, making them promising candidates for formulation in oral care products.

Objective: To evaluate the antimicrobial activity of a formulation containing Aloe Vera, chamomile, and propolis against common oral pathogens, including *Streptococcus mutans*, *Candida albicans*, *Lactobacillus* and *E. faecalis* associated with dental infections.

Methods: The formulation was prepared by diluting specified concentrations Aloe Vera, chamomile and propolis extract powders in distilled water and condensing the solution to 5 ml for further analysis. In vitro antimicrobial activity was assessed using agar well diffusion assay. The pathogens tested included *Streptococcus mutans*, *Candida albicans*, *E. faecalis*, and *Lactobacillus sp*.

Results: The formulation demonstrated significant antimicrobial activity against all tested oral pathogens, with the strongest effects observed against Lactobacilli. Maximum zone of inhibition (27 mm) was seen in the highest concentration (100 µg/mL) against Lactobacilli.

Conclusion: The Aloe Vera, chamomile, and propolis formulation exhibited promising antimicrobial properties, suggesting its potential as a natural alternative for oral care. The synergistic effects of the individual components may offer an effective adjunct to conventional treatments in managing oral infections and improving oral health.

Keywords: Aloe Vera, chamomile, propolis, antimicrobial activity, oral pathogens, *Streptococcus mutans*, *Candida albicans*

Introduction:

Herbs with medicinal characteristics are a valuable and effective source of treatment for a variety of diseases [1]. According to the World Health Organization (WHO), up to 80% of the world's population relies on traditional medicine for primary health care [2]. Screening for antimicrobial agents extracted from plants is a practical method for identifying natural compounds with antibacterial activity against dental infections [3]. Because of the rising prevalence of life-threatening bacterial, fungal, and viral infections, as well as their capacity to build resistance to current treatment options, there is an urgent need to discover and develop novel compounds to battle them.

The relationship between oral disorders and the activity of microbial species that comprise the oral cavity's microbiota is well documented. Oral microorganisms are to blame for two major human diseases: tooth caries and periodontal disorders [4]. Poor oral hygiene is a major risk factor for bacterial buildup and hazardous actions [5]. The use of several antimicrobial drugs has been proposed as a possible technique for reducing, controlling, and preventing the accumulation of cariogenic and periodontopathogenic microorganisms [6].

Aloe vera (Aloe barbadensis miller) is a plant in the Liliaceae family that is generally succulent and has a whorl of elongated, pointed leaves [7]. Due to the presence of two organic acids, cinnamic and chrysophanic acid, frequently known as chrysophanol, A. vera can inhibit the growth of bacteria and fungi [8]. Aloe barbadensis miller (Aloe vera) encompasses a wide range of medicinal properties, including anti-inflammatory, immunostimulatory, antibacterial, antifungal, and cell growth stimulating effects [9].

Chamomile is an herb that has been used for many years for its medicinal properties and was well known by ancient civilizations such as egyptian, greek and roman [10]. Over 120 components have been detected in the phytochemical profile of M. chamomilla essential oil and extracts [11]. Previous studies done on the effectiveness of chamomile revealed that the presence of alpha-bisanol in chamomile gives its anti-microbial properties [12].

Propolis (bee glue) is a sticky resinous substance made by honey bees from various plant sources such as leaves, flowers, and bud exudates, which is subsequently modified by bee secretions and wax [13]. Propolis' beneficial properties are mostly due to volatiles, flavonoids, and phenolic compounds, which are well known antioxidant and antimicrobial active constituents [14].

This study examines the antimicrobial activity of Aloe barbadensis miller (Aloe vera), chamomile and propolis formulation on some oral pathogens such as lactobacillus sp, E. Faecalis, streptococcus mutans and Candida albicans.

Materials and Methods:

Preparation of the formulation:

The following procedure was carried out to produce the aqueous formulation of propolis, chamomile, and aloe vera: One gm each of aloe vera, chamomile, and propolis were dissolved in 100 millilitres of distilled water and brought to a boil using a heating mantle at 40 to 45 degrees Celsius. The aqueous formulation was then condensed to 5 mL and put in the refrigerator for additional analysis after the solution had been filtered through a muslin cloth (figure 1).

Figure 1: Prepared Formulation (Aloe vera, propolis and chamomile)

Antimicrobial activity (agar well diffusion technique):

The antibacterial activity of the formulation of aloe vera, chamomile, and propolis was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were autoclaved for 15 to 20 minutes at 121°C to sterilise them. After sterilising the Petri plates, the medium was poured onto their surface and left to cool to room temperature. The bacterial suspension (lactobacillus sp., E. faecalis, streptococcus mutans, and Candida albicans) was evenly distributed over the agar plates using sterile cotton swabs. Wells 9 mm in diameter were made in the agar plates using a sterile polystyrene tip. Different concentrations of CuO NPs (25 g, 50 g, and 100 g) were then added to the wells. The control was an antibiotic (e.g., Bacteria-Amoxyrite, Fungi-Fluconazole). For fungal cultures, the plates were incubated for 24 and 48 hours at 37°C. To evaluate antibacterial activity, the diameter of the inhibitory zone around the wells was measured. After using a ruler to measure the zone of inhibition's diameter and recording it in millimetres (mm), the zone of inhibition was computed.

Results:

The Figure 2 shows the antimicrobial activity of the prepared formulation against lactobacillus sp, E. Faecalis, streptococcus mutans and Candida albicans. The antimicrobial susceptibility test showed the oral commensals were susceptible to aloe vera, chamomile and propolis formulation by having a clear zone of inhibition.

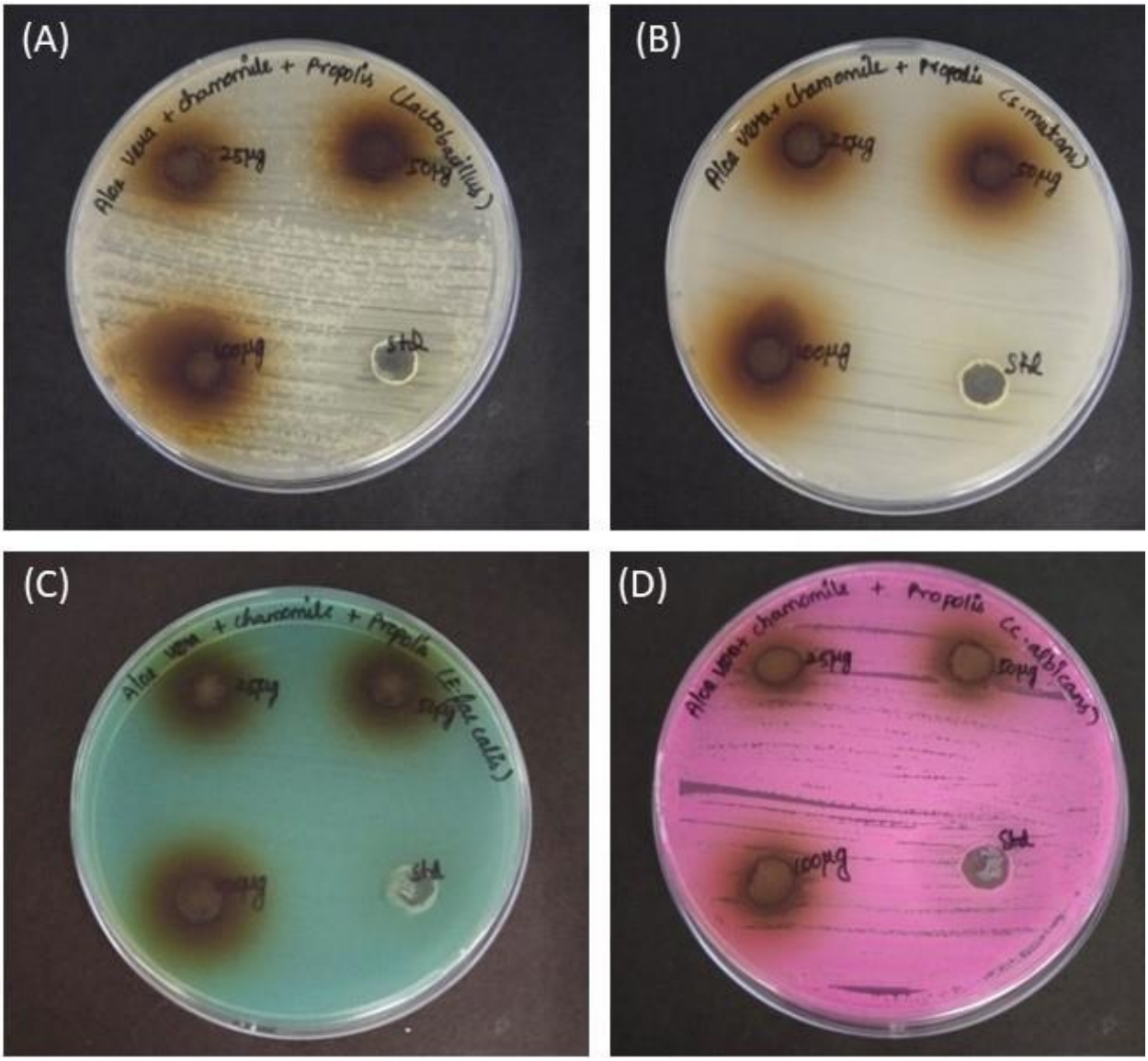

Figure 2: Zones of inhibition of the formulation against various pathogens

The zone of inhibition of the formulation against all the 4 pathogens was greater than the control used (figure 2). The inhibition zone values were double the times more than the inhibition zones found in the control. The lowest value of inhibition for the formulation was 12 mm seen against candida species, whereas the lowest value of inhibition found in the control was 9 mm.

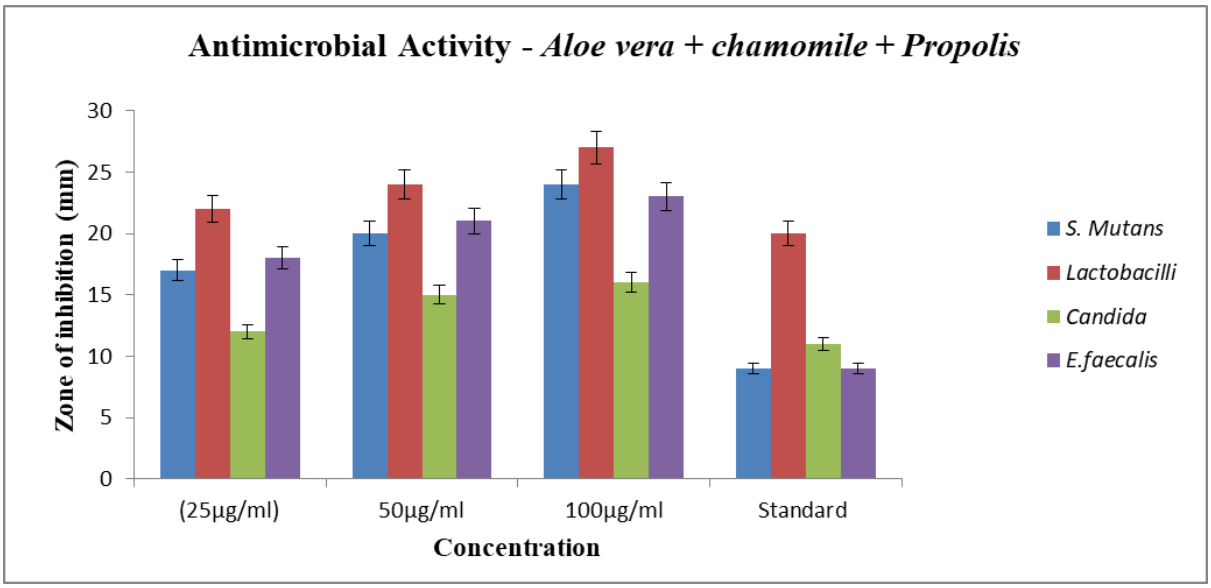

Figure 3: Antimicrobial activity of the formulation

Maximum antibacterial activity was seen in the highest concentration (100 µg/mL) in each type of microbes that were used. Lactobacilli was the most affected than the rest (27 mm). Better antimicrobial properties were observed as the zone of inhibition increased with the concentration of the formulation (figure 3 and table 2).

Microorganism	(25µg/ml)	50µg/ml	100µg/ml	Standard
S. Mutans	17	20	24	9
Lactobacilli	22	24	27	20
Candida	12	15	16	11
E.faecalis	18	21	23	9

Table 1: Zones of inhibition in millimeters(mm) against the oral pathogens

Discussion:

The prepared formulation produced a zone of inhibition of 27 mm at a concentration of 100 µg/mL for the lactobacilli sp. which was the highest zone of inhibition seen compared to the

other microbes. The lowest zone of inhibition of 16 mm at a concentration of 100 µg/mL was exhibited for the candida albicans.

In a study conducted by Jain et al, the antibacterial activity of aloe vera extract was evaluated against E. faecalis, E. coli and Staphylococcus aureus and the zone of inhibition was significantly high for all 3 oral microbes [15]. The zone of inhibition for E. faecalis was 21 mm whereas our formulation created a zone of inhibition of 23 mm at highest concentration of 100 µg/mL. Given that *E. faecalis.* bacterium has a high tendency to penetrate dentinal tubules to create biofilms, it is resistant to the conditions established by the dental instrument in the canal, the use of cleansing agents, and the use of intracanal medication such as calcium hydroxide [16].

Aloe vera gel and propolis extract were comparatively evaluated for their antimicrobial properties in a study conducted by Ehsani et al [17] both aloe vera and propolis showed significant antibacterial effects on e. Faecalis, S. Aureus and S. mutans. The zone of inhibition was significantly high than chlorhexidine which was the control that was used in the study.

In a study conducted by [18], the antibacterial effect of 3 mouthwashes (aloe vera-green tea, chamomile and chlorhexidine) were separately evaluated on five oral pathogens (Streptococcus Oralis, Streptococcus sanguis, and Streptococcus mutans, Porphyromonas gingivalis and Eikenella corrodens). Among the three mouthwashes, CHX has significantly higher antimicrobial activity when compared to the other two mouthwashes but all the three mouthwashes had significant antimicrobial effect against all the five oral pathogens.

Aloe vera was also effective in inhibiting the growth of cariogenic bacteria in a study conducted by [19]. S. Mutans was identified as the microbe that was most sensitive to aloe vera gel with a minimum inhibitory concentration of 12.5 µg/ml.

Study by Gupta et al. (2014) [15] highlighted the antibacterial effects of Aloe Vera against common oral pathogens like *Streptococcus mutans* and *Candida albicans*, emphasizing its potential for oral health. Similarly, Chamomile, as noted by Shekar et al. [20], exhibits anti-inflammatory and antimicrobial properties, which have been proven effective against various oral microorganisms. Propolis, extensively studied by Bankova et al. [21], is well-known for its potent antimicrobial and anti-inflammatory actions, which are often utilized in oral care formulations. The combination of these agents, as discussed by Ríos et al. [22], could offer a synergistic effect, potentially enhancing antimicrobial efficacy in managing oral infections. In recent years both carious and non-carious diseases occur at an early age accompanied by various other risk factors [23]. This research, therefore, builds on the growing body of evidence supporting the use of natural substances for oral health care, while aiming to assess their collective impact against oral pathogens in a formulation.

The antimicrobial property of the Aloe Vera, chamomile, and propolis formulation against oral pathogens is driven by the synergistic action of the bioactive compounds present in each

ingredient, which collectively target various microbial processes. Aloe Vera contains compounds like anthraquinones and saponins, which have been shown to disrupt microbial cell membranes, inhibit protein synthesis, and reduce biofilm formation, particularly against *Streptococcus mutans* and *Candida albicans* [15]. Chamomile, rich in flavonoids such as apigenin, has strong anti-inflammatory and antimicrobial effects, working by disrupting the cell wall integrity of bacteria and fungi, thus preventing their proliferation [23]. Propolis, which contains phenolic acids, flavonoids, and essential oils, exhibits potent antimicrobial action by interfering with microbial cell membrane function, inhibiting enzymes crucial for cell wall synthesis, and disrupting metabolic processes [24]. The combination of these bioactive compounds not only directly inhibits the growth of oral pathogens but also reduces inflammation, contributing to a dual action of antimicrobial and healing effects. This formulation, therefore, acts through multiple mechanisms; membrane disruption, enzyme inhibition, and biofilm prevention offering a comprehensive strategy to combat oral infections.

Conclusion:

The Aloe Vera, chamomile, and propolis formulation demonstrated significant activity against common oral pathogens such as *Streptococcus mutans*, *Candida albicans*, *E. faecalis*, and *Lactobacillus sp*. The synergistic effects of the bioactive compounds present in these natural ingredients resulted in enhanced antimicrobial efficacy. These findings suggest that the formulation could serve as a viable natural alternative to conventional oral care products, offering a promising adjunct in the prevention and management of oral diseases. Further studies, including clinical trials, are necessary to confirm its safety and efficacy in real-world applications.

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