Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE DOI: 10.47750/jptcp.2023.30.16.023

Preparation of mouth rinse using poly herbal formulation (Neem, White tea, cinnamon and Basil) and evaluation of antimicrobial and anti inflammatory properties

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Submitted: 29 April 2023; Accepted: 14 May 2023; Published: 23 June 2023

ABSTRACT

Introduction: Plaque buildup and oral bacteria are the key risk factors for a variety of orodental infections, thus focusing on them can be a successful strategy for treating these conditions. Due to many negative effects of conventional forms of treatment, herbal extracts have recently attracted a lot of attention.

Aim: The present study was conducted to compare the antibacterial and anti inflammatory of a new herbal combination with commercially available mouthwash against variety of microbes.

Materials And Methods: White tea, cinnamon, tulsi, and NWCT Neem were all combined into an aqueous extract. Researchers looked at the herbal extract's antimicrobial effects on the strains of Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, and Candida albicans. For this experiment, MHA agar was used to identify the zone of inhibition. The albumin denaturation assay was used to examine anti-inflammatory efficacy.

Results: 100ul of aqueous NWCT extract shows stronger antimicrobial activity against Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, and Candida albicans. 50ul of aqueous NWCT extract shows significant anti-inflammatory effects.

Conclusion: Alternatives from the herbal world may show to be a reliable and secure therapy option.

Keywords: Neem, Cinnamon, White tea, Tulsi, mouth rinse, mouthwash, Herbal rinse

INTRODUCTION

Dental illnesses like dental caries can start and progress because of salivary microfloras like Streptococcus mutans and other risk factors. One such element that puts a person at risk for periodontal disease as well as dental caries is plaque buildup. Anti inflammatory and antimicrobial medicines that target these predisposing factors thus have a substantial role in the prevention of certain oral disorders and significantly improve a person's oral health.(1,2)

Chlorhexidine has been the preferred mouthwash for a wide range of oral hygiene products owing to its impressive therapeutic effect; however, due to its numerous side effects, including taste alteration, supragingival calculus formation, and desquamation of the oral mucosa, its use in children has been constrained (3-5). Adherence to the polyphenolic and tannin groups of liquids such as tea and coffee also contributes to extrinsic staining. Therefore, alternative herbal extract-based agents are of special interest. Various herbal extracts, such as neem,white tea, chamomile, and tulsi, are known to provide therapeutic benefits in the oral cavity when used topically (6-8)

Neem, also known as Azadirachta indica, is a plant often found in India, Pakistan, Bangladesh, and Nepal and offers therapeutic benefits for a number of ailments. It belongs to the Meliaceae family. It contains a variety of different constituents, such as nimbin, nimbidin, nimbolide, and limonoids, and these kinds of substances play a part in the treatment of diseases through the manipulation of various genetic pathways and other processes. The first polyphenolic flavonoids isolated from neem leaves were quercetin and β -sitosterol, which were also recognized to have antifungal and antibacterial properties (9-11)

White tea is one of many types of tea that typically uses young or minimally processed Camellia sinensis plant leaves. White tea has mineral and antibacterial properties that shield the body from the development of illnesses. It includes various concentrations of catechins, a subset of polyphenols, and a group of phytonutrients known as polyphenols. Fluoride, flavonoids, and other tannins are also found in white tea. This is responsible for the many advantages offered by white tea. The tea catechins showed the strongest antibacterial activity. The growth of Helicobacter pylori is inhibited by tea catechins and polyphenols such as caffeic and gallic acids (12-16)

The Lauraceae family includes a tree in tropical medicine known as cinnamon (Cinnamomum zeylanicum and Cinnamon cassia). Cinnamon is one of the most significant spices that people use daily. In addition to essential oils, cinnamon also contains compounds such as cinnamaldehyde, cinnamic acid, and cinnamate. In addition to being an antioxidant, anti-inflammatory, antidiabetic, antibacterial, anticancer, lipidlowering, and cardiovascular disease-lowering agent, cinnamon has been proven to have actions against neurological diseases such as Parkinson's and Alzheimer's disease.(17-19)

Ocimum sanctum, known as tulsi, is an aromatic shrub that is indigenous to the tropics of the eastern hemisphere and is a member of the Lamiaceae (Tribe ocimeae) family of basil. It is thought to have come from north-central India.Numerous in vitro, animal, and human studies have investigated the therapeutic effects of tulsi. These studies demonstrate that tulsi has a diverse range of beneficial effects, including antimicrobial (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, and anthelmintic), mosquito repellent, antioxidant, anti-cataract, anti-inflammatory, chemopreventive, radioprotective, hepatoprotective, neuroprotective, cardioprotective, and anti-diabetic effects (20,21) Therefore, this study aimed to assess the antiinflammatory and antibacterial properties of this new herbal formulation against various oral microbes.

MATERIALS AND METHODS Extract Preparation

Neem, cinnamon, white tea, and tulsi were dried and powdered, and the aqueous extract was prepared using a Soxhlet apparatus for 8 h using a rotary evaporator (PBV-7D).1 gm of each leaf extract was dissolved in 100 ml of distilled water separately, boiled for 10-20 minutes and filtered using Whatman filter paper. 25 ml of each solution was mixed together for a total of 100 ml of the extract.



FIGURE 1: extract preparation

Mouth Wash Preparation

The polyherbal extract was added to 9 ml of distilled water. In addition, a 0.001 g of sodium benzoate, a 0.01 g of sodium lauryl sulfate and a

0.3 g of sucrose were added. The prepared mouthwash was then stored at a low temperature for further analysis.

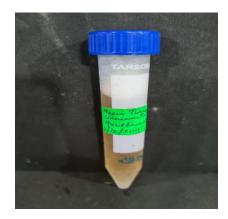
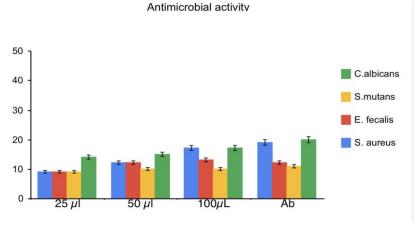


FIGURE 2: mouth wash preparation

antimicrobial activity

Antibacterial activity of the mouthwash against S. aureus, Streptococcus mutans, Enterococcus faecalis, and Candida albicans. MHA agar was used to determine the zone of inhibition. Muller Hinton Agar was prepared and sterilized for 45 minutes at 120lbs. The media were poured into sterilized plates and allowed to stabilize for solidification. The wells were cut using a well cutter and the test organisms were swabbed. Nanoparticles with different concentrations were loaded, and the plates were incubated for 24 h at 37 °C. After incubation, zones of inhibition were measured.





Anti Inflammatory Activity

Albumin Denaturation Assay

The anti-inflammatory activity of the NWCT mouthwash with various fixation $(10\mu L, 20\mu L, 30\mu L, 40\mu L, 50\mu L)$ was added to 0.45 mL bovine serum albumin (1% aqueous solution), and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. The samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min.

The samples were cooled, and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO was used as the control. Percentage of protein denaturation was determined utilizing following equation,

% inhibition= <u>Absorbance of control-</u> <u>Absorbance of sample x 100</u>

Absorbance

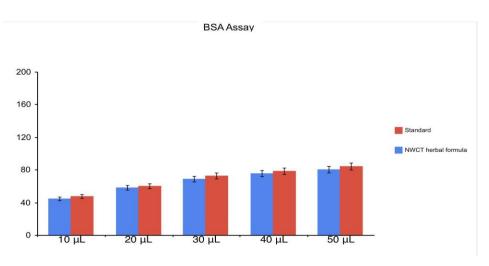


FIGURE 4: Anti inflammatory activity of NWCT

RESULTS

The highest anti- microbial activity of 17 mm as comparable to the the commercial antibiotic was observed against *Staphylococcus aureus* strain and *Candida albicans at 100ul concentration*. The next highest antimicrobial activity of 13 was showed against *Enterococcus faecalis* strain. There was also significant inhibition of 11 mm observed against *Streptococcus mutans*.

DISCUSSION

In recent years, the rise in antimicrobial resistance (AMR) has threatened humans and poses a challenge to clinicians. According to the systematic analysis of Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report:2019, around 5 million deaths in 2019 worldwide are associated with bacterial AMR. In particular, six pathogenic organisms are associated with 1 million AMR deaths, including Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa (AMR Collaborators, 2022).

Currently, researchers and clinicians are focusing on herbal medicines for treating AMR bacterial organisms. Notably, in the field of dentistry, the use of herbal extracts has increased, which have strong antimicrobial and anti-inflammatory properties that help to maintain oral hygiene and to replace synthetic drugs. Herbal plants contain various bioactive phytochemical compounds that possess strong anti-microbial, anti-inflammatory, anti-proliferative, and anti-oxidant properties; therefore, they can be used as therapeutic agents for various infectious and non-infectious diseases.(22-26)

Traditionally, medicinal plants and herbs, such as neem, cinnamon, white tea, and tulsi, are used for oral hygiene and have active phytochemical contents, including tannins, alkaloids, carbohydrates, phenols, flavonoids. and glycosides. Several studies have shown individual herbal antimicrobial and antiinflammatory activities in vitro; however, the

combination of these herbs is still unknown. The antibacterial activities of neem, cinnamon, white tea, and tulsi against Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, and Candida albicans were evaluated and compared. Numerous approaches and new technologies are available for extracting medicinally active sections of plants using specific solvents. In this study the aqueous extract of NWCT effectively inhibits the growth of Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, and Candida albicans at 100ul concentration in MIC test. Furthermore, albumin denaturation assay results revealed 50ul of aqueous NWCT extracts possess significant anti-inflammatory effects.(27-29)

Azadirachta indica (neem.) has a wide range of antibacterial activities and is currently being investigated in various domains, including dentistry, food safety, bacteriology, mycology, virology, and parasitology. Furthermore, neem extracts and phytochemicals can act against drugresistant and biofilm-forming organisms, both of which constitute a vast population of pathogens with limited treatment choices (30,31). Previously, the antibacterial activity of A. indica against common endodontic pathogens such as Enterococcus faecalis, Staphylococcus aureus, Streptococcus mutans, and Candida albicans has shown aqueous neem leaf extract, suppress their growth at higher concentration (32-34) which is in concordance with our study too. Furthermore, the methanolic extract of neem significantly inhibited microbial growth at low concentrations (35). The presence of phytocomponents, such as triterpenoids, phenolic chemicals, carotenoids, steroids, flavonoids, ketones, and the tetra-triterpenoid azadirachtin, might be attributed to its antibacterial activity against pathogens (36,37).

Ocimum sanctum L (Tulsi) also has strong antimicrobial activity, and various parts of the tulsi plant have been traditionally used to treat asthma, bronchitis, malaria, and chronic fever. Tulsi contains eugenol (l-hydroxy-2-methoxy-4allylbenzene), which exhibits antimicrobial properties. A study by Chandrappa et al. demonstrated that Tulsi has significant inhibitory effects against E. faecalis, which is equal to that of 2% chlorhexidine gluconate when compared in vitro (38,39). Several studies have suggested that tulsi has strong antimicrobial activity against various pathogens, including oral pathogens (40)

Similarly, several studies have shown that cinnamon and white tea extracts have strong anti-inflammatory, antimicrobial, and antioxidant properties. Cinnamon is commonly used in dental medicine and contains active bioactive components such as cinnamaldehyde, eugenol, phenol, and linalool. These components significantly inhibit the growth of dental caries microbes, act as antifungal and antimicrobial agents against endodontic pathogens, and reduce gingival inflammation (40-42). An in vivo study also suggested that cinnamon aqueous extract has anti-inflammatory properties that suppress TNF- α and IL-6 levels in LPS-induced mouse serum (43). White tea is a common beverage that also has therapeutic potential, and white tea mouth rinse can be used as an antiplaque agent. Tea leaves contain a variety of chemical substances, including polyphenols, caffeine, theophylline, theobromine, and other methylxanthines, lignin, organic acids, chlorophyll, free amino acids, and a number of flavor-rich substances. (44,45). A randomized clinical trial revealed that white tea extracts showed strong antibacterial and antiinflammatory properties, which effectively reduced bacterial growth in samples with periodontal infection (46). It can be used as a mouth rinse for periodontal disease and gingivitis; however, the efficacy of a single white tea extract is less than that of chlorohexidine (47,48). These results indicated that each plant possesses strong antimicrobial and antiinflammatory properties. Therefore, the combination of our NWCT extract could replace the synthetic mouthwash of chlorohexidine gluconate in dentistry.

CONCLUSION

In this study, we demonstrated that NWCT has strong antimicrobial activity against dental pathogens, and possesses anti-inflammatory properties. Furthermore, our findings suggest that NWCT-based toothpaste, mouthwash, or gel can lower S. aureus, S. mutans, E. faecalis, and C. albicans in the mouth and that the gel formulation can reduce plaque and gingivitis.

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