Preparation of mouthwash by *Saccharomyces cerevisiae* and triphala: alternative to chlorhexidine gluconate

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Abstract- *Pseudomonas aeruginosa* is considered as a reservoir of oral cavity. This bacterium is present in nosocomial infections with high morbidity and mortality. This bacterium is an initial step in pathogenesis of pulmonary infection in patients with cystic fibrosis. When these species interact with other species of periodontal pathogens has greater chances of having aggressive periodontitis. Chlorhexidine gluconate is been widely used in many mouthwashes and is known for its antibacterial activity. It has certain side effects such as staining, alteration in taste, tartar formation. The alternative to Chlorhexidine is triphala which helps in maintaining oral health and helps prevent periodontitis. In previous studies it was been observed that triphala is more effective than Chlorhexidine. Moreover, Chlorhexidine treats gingivitis, not periodontitis, whereas triphala fights against both gingivitis as well as periodontitis. *Saccharomyces cerevisiae*, non-pathogenic yeast, is a potential probiotic. It has significant antibacterial activity and immunomodulatory activity. Triphala preparation is made from Indian gooseberry (*Emblica officinalis*), belleric myrobalan (*Terminalia bellirica*) and black myrobalan (*Terminalia chebula*). It is an ancient herbal remedy with antioxidant, anti-inflammatory and antibacterial effects. It improves oral health by preventing plaque, gingivitis, heals mouth sores. It shows antibacterial activity except probiotic organisms.

In this study, effect of triphala and *S. cerevisiae* against *P. aeruginosa* was evaluated by agar-well diffusion method. Triphala solution and *S. cerevisiae* effectively showed antimicrobial activity against *P. aeruginosa* individually. Therefore, triphala and *S. cerevisiae* together were tested at various concentrations against *P. aeruginosa*. The result showed the clear zone of inhibition showing successful inhibition of *P.aeruginosa*. Therefore, mouthwash that contains triphala and *S. cerevisiae* can be efficiently use for maintaining oral health and this mouthwash can be alternative to chlorhexidine gluconate mouthwashes and triphala can be a promising therapeutic agent in treatment of periodontitis and gingivitis without any side effects on long term use.

Keywords*- *P. aeruginosa*, periodontitis, gingivitis, chlorhexidine gluconate, triphala, *S. cerevisiae

I. INTRODUCTION

Mouthwash, mouth rinse, oral rinse, or mouth bath is a liquid which is held in the mouth passively or swilled around the mouth by contraction of the perioral muscles. Mouthwashes combine ingredients to treat a variety of oral conditions, it has no standard formulation, so its use and recommendation involve concerns about patient safety. It is been stated that mouthwashes kill the bacterial plaque that causes cavities, gingivitis, and bad breath. However the use of mouthwash does not eliminate the need for both brushing and flossing (Gunsolley, 2006). Minor and transient side effects of mouthwashes are very common, such as taste disturbance, tooth staining, sensation of a dry mouth, etc. Soreness, ulceration and redness may sometimes occur (e.g., aphthous stomatitis or allergic contact stomatitis) if the person is allergic or sensitive to mouthwash ingredients, such as preservatives, colouring, flavours and fragrances.

Mouthwashes that contain chlorhexidine gluconate are been widely used. It is a prescription germicidal mouthwash that decreases bacteria in your mouth. Chlorhexidine is available in the United States under the brand names: Paroex (GUM), Peridex (3M), PerioGard (Colgate). This mouthwash is an oral solution that contains 0.12% chlorhexidine gluconate. It targets bacteria directly on contact. Dentist prescribes it to treat the inflammation, swelling, and bleeding of gingivitis, but this chlorhexidine gluconate has some limitations which was observed by many researchers. Limitations such as staining, alteration in taste, tartar formation, dosage, allergic reactions in some cases, cannot treat periodontitis, etc. In rare instances, permanent taste alteration is experienced after the treatment has run its course. The usual dosage is 0.5 fluid ounces (undiluted), twice daily for 30 seconds is been recommended if used more often can cause serious damage. Moreover, pregnant or planning on becoming pregnant women it is recommended for not using this mouthwash. It isn't approved for uses by children under the age of 18. Chlorhexidine treats gingivitis, not periodontitis. Separate treatment for periodontitis is needed. Chlorhexidine might even make gum problems like periodontitis worse (Frothingham & Carter, 2018). It was observed that there was lower saliva and plasma nitrite concentrations were found after using chlorhexidine mouthwash, followed by a trend of increased systolic blood pressure (Bescos et al., 2020). Overall, it was demonstrated that mouthwash containing chlorhexidine is associated with a major shift in the salivary microbiome, leading to more acidic conditions and lower nitrite availability in healthy individuals and this mouthwash cannot be recommended for daily use as it has various limitations.

Alternative to chlorhexidine gluconate is povidone iodine, triphala, alcohols, benzethonium chloride, benzalkonium chloride, parachlorometaxylenol (PCMX), etc. Every chemical has certain side effects which can prove to be dangerous, whereas triphala is ayurvedic, it can be used as mouthwash alternative to chlorhexidine. It was stated that the effect of Triphala and the Chlorhexidine mouthwash against oral pathogens is almost the same (Bajaj & Tandon, 2011), even it was also been found that the effect of Triphala on dental plaque, gingival inflammation, and microbial growth shows better results than chlorhexidine (Rana et al.,2016). Therefore, triphala can be used as mouthwash which is alternative to chlorhexidine. Studies have shown that Triphala has an anti-microbial, antibacterial, antioxidant, anti- collagenase and anti-inflammatory properties in their formulation which is of wide spread interest in dentistry. It can prevent free radicals from causing cell damage. Triphala preparation is made from Indian gooseberry/ Amla (*Emblica officinalis*), belleric myrobalan/ bibhitaki (*Terminalia bellirica*) and black myrobalan/ hartaki (*Terminalia chebula*). It has abundant therapeutic values. Triphala has shown positive effect on Plaque and Gingivitis as well as periodontitis ("ROLE OF TRIPHALA MOUTHWASH IN GINGIVITIS AND PERIODONTITIS- A NARRATIVE REVIEW", 2020). It was been observed that triphala shows inhibitory effect on microbial counts except *Lactobacillus* and other probiotic organisms.

Probiotics are a group of organisms those confer health benefit to consumers. To be used as probiotic, an organism should possess several attributes such as adhesive ability, acid and H_2O_2 production ability, bile tolerance and significant antibacterial activity and immunomodulatory activity and must be non-pathogenic. *Saccharomyces cerevisiae* is unicellular yeast and one of the most explored organisms in terms of industrial applications and genetic studies. Several previous studies showed that members of *Saccharomyces* genus possess anti-bacterial and probiotic properties. Antibacterial capability of *S. cerevisiae* might be due to production of extracellular protease, secretion of inhibitory proteins, stimulation of immunoglobulin A, acquisition and elimination of secreted toxins, killer toxins, sulfur dioxide etc (Fakruddin et al., 2017).Several studies have also been reported with the use of yeasts (*S. boulardii* or *S. cerevisiae)* as a potential bio therapeutic agent (probiotic) for the treatment of microbes associated diarrhoea and colitis (Fakruddin et al., 2017). Therefore, this organism can be used with triphala in preparation of mouthwash, as triphala doesn't inhibit probiotic organisms, which was been reported in earlier studies (Rana et al., 2016).

Periodontitis, also called gum disease, is a serious gum infection that damages the soft tissue and, without treatment, can destroy the bone that supports your teeth. Periodontitis can cause teeth to loosen or lead to tooth loss (Periodontitis - Symptoms and causes, 2022). Periodontitis has a multifactorial etiology involving group

or specific group of microorganisms, host response, local, environmental and genetic factors. Chemotherapeutic agents, respective procedures, regenerative procedure, plastic surgery and occlusal therapy are the following courses of treatment suggested for periodontitis based on its severity. Systemic antibiotic was traditionally used, but patient compliance to dose; varying absorption in gastrointestinal tract; raising antimicrobial resistance were some limitations of this treatment approach, whereas local antibiotics had only minimal differences when compared to scaling and root planning. Chemical agents (chlorhexidine, triclosan, cetylpyridinium chloride) have only limited value. Due to its multifactorial etiology, it becomes a formidable task for dentist to provide treatment for periodontitis ("ROLE OF TRIPHALA MOUTHWASH IN GINGIVITIS AND PERIODONTITIS- A NARRATIVE REVIEW", 2020).

Pseudomonas aeruginosa is a ubiquitous Gram-negative bacterium that can act as an opportunistic pathogen frequently present in the oral and cloaca microbiota of healthy ophidians. It can cause severe clinical diseases and often shows antibiotic resistance (Sala et al., 2019). *P. aeruginosa* is present in the different oral ecologic sites (dorsum of tongue, buccal mucosa, dental plaques) (Komiyama et al., 1985). Cystic fibrosis (CF) is the most frequent lethal genetic disease. Lung destruction is the principal cause of death by chronic *P. aeruginosa* colonization. There is a high prevalence of oropharyngeal anaerobic bacteria in sputum of CF patients (Rivas Caldas et al., 2015). In previous studies it is suggest that there is possibility that oral colonization by *P. aeruginosa* may be an initial step in the pathogenesis of pulmonary infection in patients with CF (Komiyama et al., 1985) as well as it is also been observed that when these species interact with other species of periodontal pathogens has greater chances of having aggressive periodontitis. Therefore, inhibition of this organism is necessary in mouth itself, as if it is inhibited in mouth, it will not cause or prevent oral diseases as well as pulmonary infection in patients with CF.

The present study aimed to prepare a mouthwash made from triphala and *S. cerevisiae* that can effectively inhibit *P. aeruginosa* in mouth itself. This mouthwash will also show the probiotic effect. Moreover, the antimicrobial activity of *Saccharomyces cerevisiae* against pathogenic strains such as *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Streptococcus pyogenes* were determined and then the antimicrobial activity of triphala was checked against only those pathogens which were inhibited by *S. cerevisiae*. The mouthwash was prepared at specific concentration of triphala and *S. cerevisiae* which showed better antimicrobial activity against pathogen.

II. LITERATURE SURVEY

Different studies are conducted with respect to antimicrobial and antioxidant activities of *Saccharomyces cerevisiae,* which is a potential probiotic. The importance of triphala, its uses in maintaining good oral health and preventing various oral diseases and is alternative to chlorhexidine gluconate is been reported. The triphala and *S. cerevisiae* is reported to be effective against oral pathogens.

(Rivas Caldas, et al. 2015) reported that cystic fibrosis (CF) is the most frequent lethal genetic disease. Lung destruction is the principal cause of death by chronic *Pseudomonas aeruginosa* colonization. This study was carried out due to the lack of results comparing subgingival periodontal pathogenic bacteria between the oral cavity and lungs in patients with CF in relation with *P. aeruginosa* presence. A cross-sectional pilot casecontrol study was conducted. 10 CF patients with homozygous mutation (5 chronically colonized [CC] and 5 not colonized [NC]) were enrolled. *P. aeruginosa* was detected in saliva, sputum, and subgingival plaque samples by real-time quantitative PCR (qPCR). Subsequently, periodontal bacteria were also detected and quantified in subgingival plaque and sputum samples by qPCR. In CC patients, *P. aeruginosa* was recovered in saliva and subgingival plaque samples. 16 *P. aeruginosa* strains were isolated in saliva and sputum from this group and compared by pulsed-field gel electrophoresis (PFGE). Subgingival periodontal anaerobic bacteria were found in sputum samples. A lower diversity of these species was recovered in the CC patients

than in the NC patients. The presence of the same *P. aeruginosa* clonal types in saliva and sputum samples underlines that the oral cavity is a possible reservoir for lung infection.

(Souto et al., 2014) reported that *P. aeruginosa* and *Acinetobacter spp*. are important pathogens associated with late nosocomial pneumonia in hospitalized and institutionalized individuals. These respiratory pathogens are present in oral cavity, due to poor oral hygiene and periodontal infection. This study investigated the prevalence of these organisms in subgingival biofilm and saliva of subjects with periodontal disease or health. Samples were obtained from 55 periodontally healthy (PH) and 169 chronic periodontitis (CP) patients. DNA was obtained from the samples and detection was carried out by multiplex and nested PCR. *P. aeruginosa* and *Acinetobacter spp.* were detected in 40% and 45% of all samples, respectively. No significant differences in the distribution of these microorganisms between men and women, subgingival biofilm and saliva samples, patients < 35 and > 35 years of age, and smokers and non-smokers were observed regardless periodontal status ($p > 0.05$). In contrast, the frequencies of these organisms in saliva and biofilm samples were significantly greater in CP than PH patients ($p < 0.01$). Smokers presenting *P. aeruginosa* and high frequencies of supragingival plaque were more likely to present CP than PH. *P. aeruginosa* and *Acinetobacter spp.* are frequently detected in the oral microbiota of CP. Poor oral hygiene, smoking and the presence of *P. aeruginosa* are strongly associated with periodontitis was concluded.
(Bescos, R., et al. 2020) reported the investigation of the effect of 7-day use of chlorhexidine (CHX) mouthwash on the salivary microbiome as well as several saliva and plasma biomarkers in 36 healthy individuals. They rinsed their mouth (for 1 min) twice a day for 7 days with a placebo mouthwash and then repeated this protocol with CHX mouthwash for a further 7 days. Saliva and blood samples were taken at the end of each treatment for analysis. CHX significantly increased the abundance of *Firmicutes* and *Proteobacteria*, and reduced the content of *Bacteroidetes*, TM7, SR1 and *Fusobacteria*. This shift was associated with a significant decrease in saliva pH and buffering capacity, accompanied by an increase in saliva lactate and glucose levels. Lower saliva and plasma nitrite concentrations were found after using CHX, followed by a trend of increased systolic blood pressure. Overall, this study demonstrates that mouthwash containing CHX is associated with a major shift in the salivary microbiome, leading to more acidic conditions and lower nitrite availability in healthy individuals.

(Fakruddin et al., 2017) reported that *S. cerevisiae* could be potential as probiotic to be used therapeutically. Their study was based on antimicrobial and antioxidant activities of *Saccharomyces cerevisiae* IFST062013 which is a potential probiotic, was isolated from fruit. The isolate was tolerant to wide range of temperature, pH, high concentration of bile salt and NaCl, gastric juice, intestinal environment, α-amylase, trypsin and lysozyme. It can produce organic acid and showed resistance against tetracycline, ampicillin, gentamycin, penicillin, polymixin B and nalidixic acid. The isolate showed moderate anti-microbial activity against bacteria and fungi and cell lysate showed better antimicrobial activity than whole cell and culture supernatant. The isolate did not induce any detectable change in general health of mice upon oral toxicity testing and found to be safe in mouse model. The isolate improves lymphocyte proliferation and cytokine production in treated mice.

(Bhuvaneswari, M., et al. 2020) reported the effectiveness of Triphala against gingivitis and periodontitis. Triphala is a mixture of three myrobalan known as *Emblica officinalis* (Amalaki), *Terminalia bellerica* (Bibhitaki), and *Terminalia chebula* (Haritaki). Results showed that Triphala possess varying therapeutic potentials. Particularly antimicrobial and anti-inflammatory, anti-collagenase and anti-oxidant properties are of greater importance in dentistry. Various studies showed that Triphala is equally as effective as a standard chemotherapeutic agent that is chlorhexidine in treatment and prevention of oral diseases and even triphala showed better results than chlorhexidine in treatment and prevention of gingivitis and periodontitis. Therefore, it was concluded that Triphala can be a promising therapeutic agent in treatment of gingivitis and periodontitis with no side effects on long term use.

(Singh Rana, D., et al. 2016) reported that Triphala is among the most common formula used in Traditional

Ayurvedic Medicine. This preparation is composed of 3 equal number of herbal fruits: *Terminalia chebula*, *Phyllanthusemblica*, and *Terminalia bellirica*. Triphala has been proven to have antibacterial, antiviral, and antifungal actions. It was also said to possess antihistamine, anti-inflammatory, antioxidant, antitumor, blood pressure lowering, cholesterol lowering, digestive, diuretic, and laxative properties. Chlorhexidine, a cationic bisbiguanide with a very broad antimicrobial spectrum is used as counter mouth rinse. However; chlorhexidine has several side effects, such as staining and taste alteration, which limit its long-term use. Therefore, chlorhexidine is used as a positive control in many clinical trials of new mouth rinse formulations and is considered the gold standard. The aim of this review was to evaluate the efficacy of triphala and chlorhexidine mouth rinse against dental plaque, gingival inflammation, and microbial growth and the result showed that triphala showed better results in preventing periodontitis pathogens than chlorhexidine. Therefore, triphala can be effective alternative to chlorhexidine in mouthwash was concluded.

(Biradar et al., 2008) reported that triphala Mashi is an ayurvedic formulation that was prepared in the lab. Aqueous and alcoholic extracts of both Triphala and Triphala Mashi were used, to evaluate antimicrobial activity. Comparative phytochemical profile of Triphala and Triphala Mashi was done by preliminary phytochemical screening, total phenolic content and thin layer chromatography (TLC). Antimicrobial activity includes isolation of pathogens from clinical samples, its characterization, testing its multiple drug resistance against standard antibiotics and antimicrobial activity of aqueous and alcoholic extracts of both Triphala and Triphala Mashi against the organisms *(E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus*) by using agar gel diffusion method. Triphala Mashi containing phenolic compounds, tannins exhibited comparable antimicrobial activity in relation to Triphala against all the microorganisms tested. It inhibits the dose-dependent growth of Gram-positive and Gram-negative bacteria. In conclusion, it appears that Triphala Mashi has non-specific antimicrobial activity.
(Omran et al., 2020) reported the prevalence of nosocomial infections due to multidrug resistant (MDR) bacterial strains is associated with high morbidity and mortality. Triphala, an Ayurvedic formula composed of three different plants: *Terminalia chebula*, *Terminalia bellirica* (Gaertn.) Roxb. (Combretaceae), and *Phyllanthus emblica L.* (Phyllanthaceae), is used widely for various microbial infections. Microwave-assisted extraction (MAE) was shown to be the most efficient method based on yield, extraction time, and selectivity. The Triphala hydroalcoholic extract (TAE) has been chemically characterized with spectroscopic and chromatographic techniques. TAE was evaluated alone or with carvacrol. Different drug formulations including cream and nanoemulsion hydrogel were prepared to assess the antimicrobial activity against selected microorganism strains including Gram positive and Gram-negative bacteria and fungi. They used a lipophilic oil of carvacrol (5 mg/mL) and a hydrophilic TAE (5 mg/mL) ingredient in a dosage form. Two solutions were created: hydrogel containing nanoemulsion as a lipophilic vector dispersed in the gel as a hydrophilic vehicle and a cream formulation, an oil-in-water emulsion. In both cases, the concentration was 250 mg of active ingredient in 50 mL of final formulation. The formulas developed were stable from a physical and chemical perspective. In the nanoemulsion hydrogel, the oil droplet size ranged from 124 to 129 nm, with low polydispersity index (PdI) 0.132 ± 0.013 and negative zeta potential -46.4 ± 4.3 mV. For the cream, the consistency factor (acetyl alcohol and white wax) induced immobilization of the matrix structure and the stability. Triphala hydroalcoholic extract in drug nanoformulation illustrated might be an adjuvant antimicrobial agent for treating various microbial infections.

Considering all the fact, periodontics which is gum disease cannot be treated by chlorhexidine mouthwash and the causative agent is *P. aeruginosa*. Moreover, this chlorhexidine mouthwash also has certain side effects. Therefore, triphala can be alternative to chlorhexidine as it also shows better effectivity against oral pathogens and periodontitis causing agents. Triphala has antimicrobial activities except probiotic organisms, so *S. cerevisiae* which shows antimicrobial and antioxidant activities, can be used for the preparation of mouthwash so the mouthwash will have ayurvedic as well as probiotic effect and can successfully prevent and inhibit periodontitis causing agent i.e., *P. aeruginosa.*

III. METHODOLOGY

1) Isolation of test organisms and *Saccharomyces cerevisiae*

The test organisms *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes* and *Saccharomyces cerevisiae* were isolated. These were subculture on specific media, procured from HiMedia Laboratory Pvt. Ltd., Bombay, India, recommended for different microorganisms such as Brain heart infusion agar, BHI (*S. pyogenes*), Nutrient agar (*S. aureus*) and Sabouraud dextrose agar, SAB (*Saccharomyces cerevisiae* and *Candida albicans*) and King's B, KB (*Pseudomonas aeruginosa*) and incubated aerobically at room temperature. 2) Identification test of *Pseudomonas aeruginosa*

The test which was performed for identification were Gram nature (Tankeshwar, 2022), Biochemical Test which include- Catalase test, Methyl red test, Voges-Proskauer test, Citrate test ("Citrate Test - Medium, principle and procedure for citrate utilization test", n.d.), Nitrate reduction (Tankeshwar, 2022), Pigment production which was tested on King's B media. For observing pigment production, the culture was streaked on the plate and incubated at 37° C for 24hrs and Fermentation of sugar test i.e., Mannitol and lactose was performed.

3) Preparation of triphala powder and its solution

Triphala is a combination of three fruits named Harad (*Terminalia chebula*), Baheda (*Terminalia bellirica*) and Amla (*Emblica officinalis*). For the preparation of Triphala powder the ingredients was taken in the order of 1:2:4 respectively. 5grams of Harad, 10grams of Baheda and 20grams of Amla powder were mixed properly, and then 1gram of this mix powder is added in 250ml of distilled water. Then this solution was boiled and reduced to 62.5 ml and then was cooled. After cooling, it was filtered with a strainer and kept preparation in a clean, sterilized bottle.

4) Antimicrobial activity of *Saccharomyces cerevisiae* against pathogens

Agar well method was carried out to check the antimicrobial activity of *Saccharomyces cerevisiae* against pathogenic strains such as *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Streptococcus pyogenes.* In this method 1ml of these cultures (OD=0.6) were mixed in 15ml of molten Mueller Hinton (MH) agar butt respectively. Then it is poured on the sterile plates. After solidifying the media, the well was punch using sterile cork borer. Then 10-fold dilutions till 10^{-3} of *S. cerevisiae* whole cell suspension (OD=0.6) and supernatant of culture suspension were prepared. For supernatant the culture suspension was centrifuged at 2000rpm for 10mins. In 1st MH plate, in the well the 100 µl *S. cerevisiae* (whole cell suspension) undiluted (UD) and diluted samples (till 10^{-3}) were inoculated and in 2nd MH plate *S. cerevisiae* (culture supernatant) undiluted (UD) and diluted samples (till 10^{-3}) were inoculated. That is for checking antimicrobial activity against each pathogen 2 plates were prepared. Inhibitory zone of *S. cerevisiae* was checked after 24h incubation at room temperature.

5) Antimicrobial activity of triphala against *Saccharomyces cerevisiae*

For determining the antimicrobial activity of triphala against *S. cerevisiae,* agar well diffusion technique was used. In this method 1ml of *S. cerevisiae* cultures (OD=0.6) and supernatant of *S. cerevisiae* cultures suspension were mixed in 15ml of molten Mueller Hinton agar butt respectively. For supernatant the culture suspension was centrifuged at 2000rpm for 10mins. Then it is poured on the sterile plates. After solidifying the media, the well is punch using sterile cork borer. In the wells 100µl of undiluted (UD) and diluted triphala solution was inoculated. 10-fold serial dilutions up to 10^{-3} were prepared and were inoculated in the respective

wells These were done in duplicates. These plates were incubated at room temperature for 24hours. 6) Antimicrobial activity of triphala against pathogens

Pathogens which were inhibited by *Saccharomyces cerevisiae* were selected. The antimicrobial activity of triphala was checked against only those pathogens. In this method 1ml of those pathogen whole cell cultures (OD=0.6) and culture supernatant were mixed in 15ml of molten Mueller Hinton agar butt respectively. Then it was poured on the sterile plates. After solidifying the media, the well was punch using sterile cork borer. For testing antimicrobial activity of triphala against pathogens 2 sets of triphala solution were prepared. Already/firstly prepared triphala solution was labelled as undiluted (UD) and, in another set, 2ml from firstly prepared triphala solution was taken and was boiled to reduce to 1ml this solution was labelled as concentrated (Conc). These 2 sets were inoculated (100µl) into the respective wells. These were done in duplicates. These plates were incubated at room temperature for 24hours.

7) Antimicrobial activity of triphala + *Saccharomyces cerevisiae* against pathogen

The pathogens which were inhibited by triphala solution were only selected for determining antimicrobial activity of triphala + *S. cerevisiae*. In this method 1ml of those pathogen whole cell cultures (OD=0.6) or the culture supernatant were mixed in 15ml of molten Mueller Hinton agar butt. Then it was poured on the sterile plates. After solidifying the media, the well was punch using sterile cork borer. In the wells 100µl of different dilutions of triphala and *S. cerevisiae* whole cell suspension or culture supernatant were added (table 1). These were done in duplicates. These plates were incubated at room temperature for 24hours.

Wells	Triphala solution (ml)	*S. cerevisiae* (ml)
1	1	1
2	1	0.5
3	0.5	1
4	control	

Table 1: Dilutions for triphala + **IV. RESULT***S. cerevisiae* **S AND DISCUSSION**for inoculation in wells.

IV. RESULTS AND DISCUSSION

1) Identification of *P. aeruginosa*

The isolate was found to be gram negative rods (Fig 1). The catalase test was found to be positive (Fig 2). The result of the test methyl red and Voges Proskauer showed negative (Fig 3). Whereas the isolate showed positive citrate test (Fig 4) and nitrate reduction test (Fig 5). For pigment production test the test culture was streak on King's B agar plate and colour change of plate was observed. The result of isolate showed positive result as the colour of media changed to green (Fig 6). Moreover, the isolate was inoculated in the sugar medium of mannitol and lactose for testing fermentation of sugar test. The result showed positive mannitol and negative lactose (Fig 7). Therefore, from all these results of gram nature and biochemical tests we can confirm the identification of the isolate to be *Pseudomonas aeruginosa.*

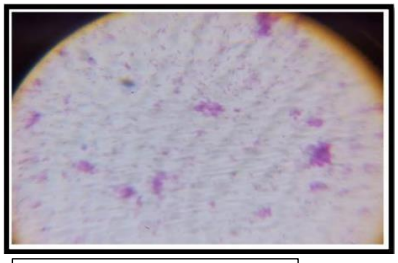


Fig 1- Gram nature

Fig 2- Catalase test

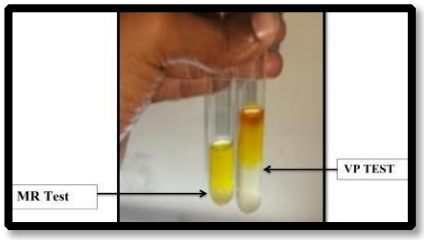


Fig 3- Methyl red and Voges Proskauer

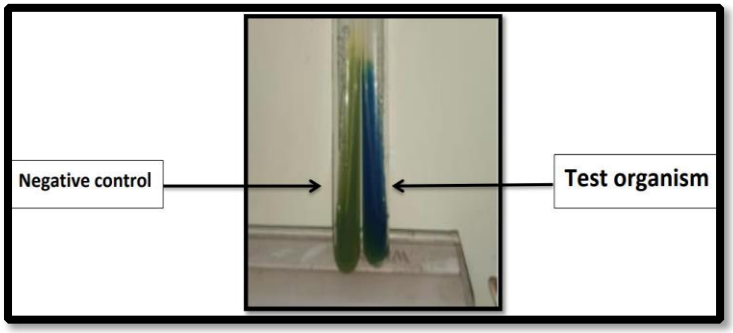


Fig 4- Citrate test

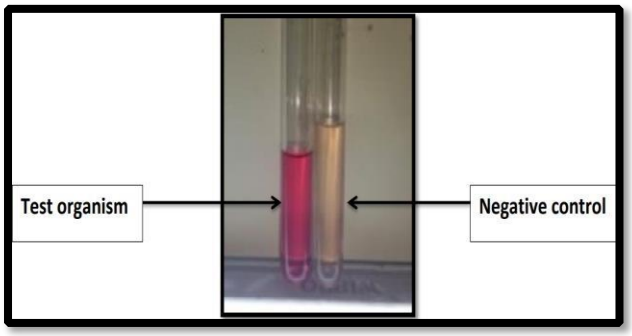


Fig 5- Nitrate test

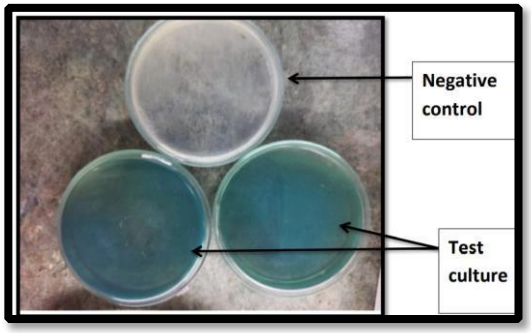


Fig 6- Pigment production

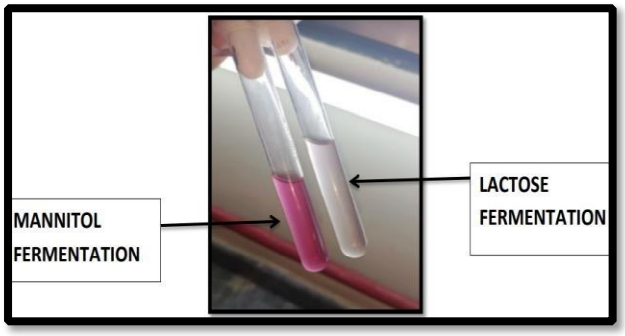


Fig 7- Fermentation of sugars – mannitol and lactose

2) Preparation of triphala powder.

Triphala powder was prepared by combining Harad (*Terminalia chebula*), Baheda (*Terminalia bellirica*) and Amla (*Emblica officinalis*). For the preparation of Triphala powder the ingredients was taken in the order of 1:2:4 respectively (Fig 8). Later on, the solution of this powder was prepared according to the given procedure and was used for further experiments.

Fig 8- Triphala powder

3) Antimicrobial activity of *Saccharomyces cerevisiae* against pathogens

The antimicrobial activity of *Saccharomyces cerevisiae* was determined against various pathogenic strains such as *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Streptococcus pyogenes*. Result showed that *S. cerevisiae* did not inhibit *Staphylococcus aureus, Escherichia coli*, and *Streptococcus pyogenes* as no inhibition zone was observed, whereas *S. cerevisiae* undiluted whole cell suspension and culture supernatant, both successfully showed antimicrobial activity against *C. albicans* (Fig 9) and *P. aeruginosa* (Fig 10), zone of inhibition is shown in table 2.

Pathogens that showed inhibition zones	*S. cerevisiae* (undiluted)	Zone of inhibition (mm)
C. albicans	Whole cell suspension	13.5
	Culture supernatant	13.0
P. aeruginosa	Whole cell suspension	15.0
	Culture supernatant	13.5

Table 2: Result showing zone of inhibition of antimicrobial activity of *S. cerevisiae* against pathogens

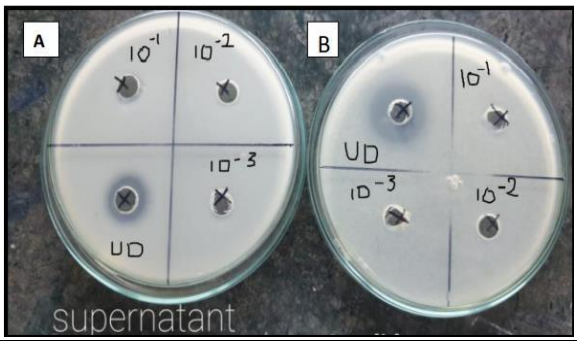


Fig 9 - Antimicrobial activity of *S. cerevisiae* against *C. albicans.* (A) Culture supernatant (B) whole cell culture.

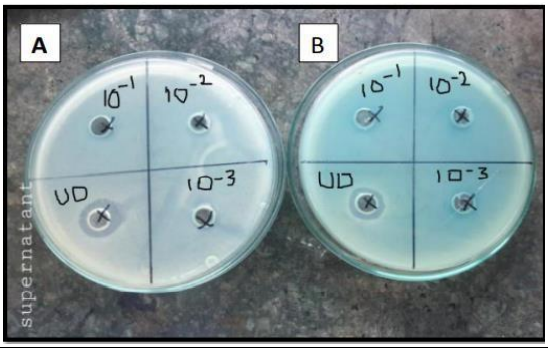


Fig 10- Antimicrobial activity of *S. cerevisiae* against *P. aeruginosa* (A) Culture supernatant (B) whole cell culture.

4) Antimicrobial activity of triphala against *Saccharomyces cerevisiae*

The antimicrobial activity of triphala against *S. cerevisiae* was determined by agar well method. Different dilutions of triphala powder were prepared. Whereas whole cell suspension and culture supernatant of *S. cerevisiae* were used. Result showed that *S. cerevisiae* was not inhibit by triphala as no inhibition zone was observed, so it was found that triphala doesn't show antimicrobial activity against *S. cerevisiae* (Fig 11).

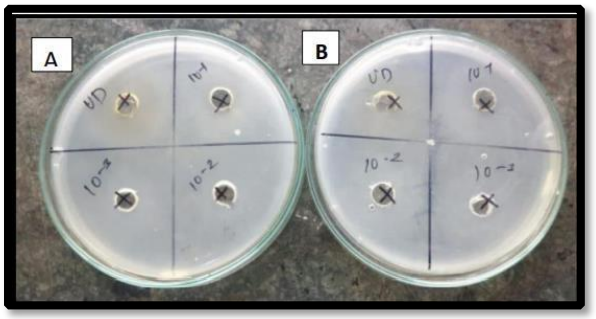


Fig 11- Antimicrobial activity of triphala against *Saccharomyces cerevisiae*. (A) Culture supernatant (B) whole cell culture

5) Antimicrobial activity of triphala against pathogens

Pathogens which were inhibited by *S. cerevisiae* that are *C. albicans* and *P. aeruginosa* were selected. The antimicrobial activity of triphala was checked against only those pathogens. Pathogens whole cell and culture suspension were taken into consideration. For testing antimicrobial activity of triphala against pathogens two sets of triphala solution were prepared. Already/firstly prepared triphala solution was labelled as undiluted (UD) and, in another set, 2ml of firstly prepared triphala solution was taken and was boiled to reduce to 1ml this solution was labelled as concentrated (Conc). The test was performed in duplicates. The result showed that triphala didn't show antimicrobial activity against *C. albicans* as no zone of inhibition was observed whereas triphala successfully inhibited *P. aeruginosa* whole cell culture and culture supernatant as well (Fig 12) and zone of inhibition is also been noted (table 3).

Triphala	*P. aeruginosa*	Zone of inhibition (mm)	Average (mm)
Set 1 Undiluted (original)	Culture supernatant	UD1= 24	24.5
		UD2=25	
	Whole cell culture suspension	UD1=25.5	26.75
		UD2=28	
Set 2 concentrated	Culture supernatant	Conc 1= 29	28.5
		Conc2=28	
	Whole cell culture suspension	Conc 1=31	31.5
		Conc2=32	

Table 3- Result of Antimicrobial activity of triphala against *P. aeruginosa*

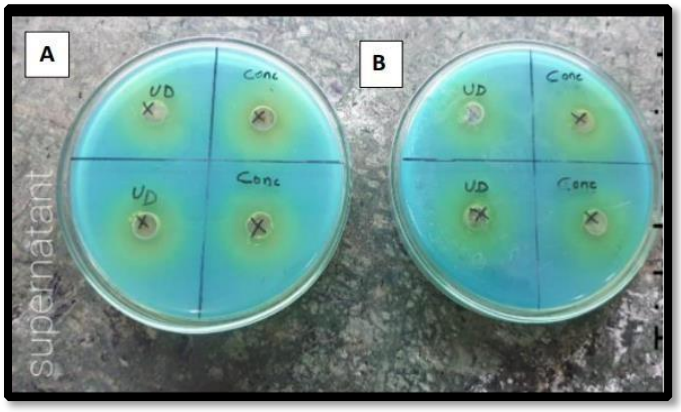


Fig 12- Antimicrobial activity of triphala against *P. aeruginosa*. (A)- Culture supernatant, (B) - whole cell suspension; UD-undiluted/original triphala solution, Conc- concentrated triphala solution.

6) Antimicrobial activity of triphala + *Saccharomyces cerevisiae* against pathogen

The pathogen which was inhibited by triphala and *S. cerevisiae* was *P. aeruginosa*. Therefore, antimicrobial activity of triphala + *S. cerevisiae* against *P. aeruginosa* was determined. As triphala was able to inhibit *P. aeruginosa* whole cell cultures (table 3) therefore 1ml of *P. aeruginosa* (OD=0.6) was mixed in 15ml of molten MH agar butt and was poured on sterile plates. After solidifying the media, the well was punch using sterile cork borer. In the wells 100µl of different dilutions of triphala and *S. cerevisiae* culture were added as shown in table 4. The whole cell culture suspension of *S. cerevisiae* was considered as it showed better zone of inhibition than culture supernatant (table 2). These were done in duplicates. These plates were incubated at room temperature for 24hours. The result showed that triphala solution and *S. cerevisiae* successfully inhibit *P. aeruginosa*. The zone of inhibition differs according to dilution (fig 13). It was observed that 1:1 ratio of triphala powder solution and *S. cerevisiae* showed greater zone of inhibition than any other dilutions, so in the mouthwash 1:1 ratio can be used (table 4).

Wells	Triphala solution (ml)	*S. cerevisiae* (ml)	Zone of inhibition (mm)
1	1	1	21
2	1	0.5	19
3	0.5	1	18
4	control		none

Table 4: Result of antimicrobial activity of triphala + *Saccharomyces cerevisiae* against *P. aeruginosa* whole cell culture suspension.

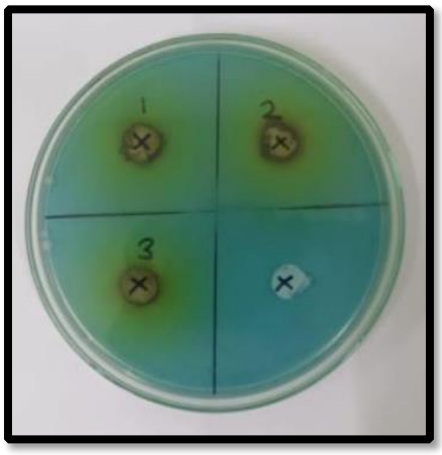
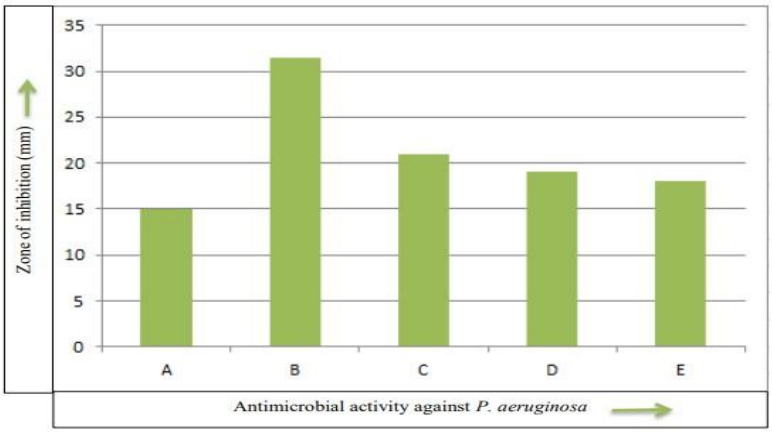


Fig 13: Antimicrobial activity of triphala + *Saccharomyces cerevisiae* against *P. aeruginosa*



Graph 1 : Comparison of antimicrobial activity against *P. aeruginosa* ; where **A**: antimicrobial activity of *S. cerevisiae*, **B**: antimicrobial activity of concentrated triphala powder solution, **C**: antimicrobial activity of 1:1 ratio of concentrated triphala powder solution and *S. cerevisiae* , **D**: antimicrobial activity of 2:1 ratio of concentrated triphala powder solution and *S. cerevisiae* , **E**: antimicrobial activity of 1:2 ratio of concentrated triphala powder solution and *S. cerevisiae*.

Graph 1 compares all antimicrobial activities which were determined in this study against oral pathogen *P. aeruginosa*. Here it was observed that alone triphala powder solution shown greater zone of inhibition (Graph 1B). So, we can conclude that triphala alone is more efficient than whole cell culture of *S. cerevisiae*, but alone triphala mouthwash will not have any probiotic effect, as probiotic mouthwash may help in maintaining healthy balance in mouth (Frisbee, 2020). Moreover, it was also been observed that antimicrobial activity of 1:1 ratio of concentrated triphala powder solution and *S. cerevisiae* showed greater zone of inhibition against *P. aeruginosa* than any other dilutions (Graph 1C). Therefore, this dilution can be used for preparation of mouthwash which will be alternative to chlorhexidine as well as will have probiotic effect. This novel mouthwash is also edible, so if mistakenly ingested, will not cause any problem as this mouthwash does not contain any chemicals. Whereas one has to also notice that when the concentration of triphala powder solution is more than *S. cerevisiae* it showed greater zone of inhibition (Graph 1D and 1E) (table 4). Therefore, triphala has more antimicrobial activity than *S. cerevisiae* against *Pseudomonas aeruginosa*.

V. CONCLUSION AND FUTURE SCOPE

This study showed that mouthwash prepared by triphala and *Saccharomyces cerevisiae* can efficiently inhibit *Pseudomonas aeruginosa*, which is an oral pathogen. The 1:1 ratio of concentrated triphala powder solution and whole culture suspension *S. cerevisiae* can be significantly used for the preparation of mouthwash as at this concentration zone of inhibition of *P. aeruginosa* was more than any other concentration. This mouthwash will have natural properties of triphala and probiotic effects of *S. cerevisiae*. Therefore, it can be efficiently use for maintaining oral health and as triphala can be a promising therapeutic agent in treatment of periodontitis and gingivitis without any side effects on long term use. Moreover, as per the previous studies,

instead of using chlorhexidine gluconate mouthwashes, this mouthwash can be used as it doesn't have any chemicals and is completely natural.

Some of future perspectives of this mouthwash are that this mouthwash may be inhibiting other oral pathogens or periodontitis causing organisms, therefore antimicrobial activity against those pathogens must be determined. Its mode of molecular actions and cytotoxic effect on human oral cancer cell lines can be determined. So, if this mouthwash is able to inhibit pathogen or viruses that can cause oral cancer e.g., Human papilloma virus, this oral cancer can be prevented. Antibiotic activity of mouthwash must be determined. Solution of triphala powder and *S. cerevisiae* can be used as natural probiotic drink as these both substances are edible, so its activity as drink must be determined.

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