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Preparation of Poly- Herbal Mouthwash and Evaluation of Antimicrobial Activity Against Common Oral Pathogens

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Abstract

Polyherbal mouthwash is an oral preparation formulated with extracts of natural ingredients such as leaves of Neem, the pericarp of Barro, the Betel plant, and the rhizome of Turmeric, along with specific antibacterial agents that show antimicrobial properties. It is applied to maintain oral hygiene, prevent bad breath, and prevent dental and periodontal disease. Polyherbal mouthwash prepared from these plants is used to study their antimicrobial effect against oral pathogens like Staphylococcus aureus, Candida albicans, Escherichia coli, Enterococcus, and Klebsiella, which are commonly found in the oral cavity. The formulation of the polyherbal mouthwash was done by the hydroalcoholic extraction method using a ratio of 1:5 of pulverized powder and solvent (70% alcohol). After the extraction, the mother liquor was evaporated using a water bath at 50°C, and the powder of the active extract of the plant was collected and stored in an airtight container at 2–8 °C. The polyherbal mouthwash was formulated in four different formulations: F1, F2, F3, and F4, in increasing concentrations, respectively. The evaluation of the antimicrobial activity of the formulated polyherbal mouthwash was done by the agar well diffusion method and compared to a commercially available mouthwash (chlorhexidine) as a standard. The zone of inhibition of bacterial growth was measured and compared to the standard. Among the four formulations, F4 had the most effective zone of inhibition: Enterococcus, C. albicans, and S. aureus were 24mm, 26mm, and 18mm, respectively, compared to the standard (chlorhexidine), which were 20mm, 26mm, and 20mm. However, the hydroalcoholic preparation of the polyherbal mouthwash was resistant to E. coli and K. pneumoniae, while the standard was effective toward these bacteria. The formulated mouthwash's physical properties (color, consistency, odor, pH, density, and viscosity) were also evaluated. The results of this study will provide

valuable information on the potential benefits of using a polyherbal mouthwash as part of a daily oral care routine

Keywords: *Azadirachta indica, Terminalia bellirica, Piper betle, Curcuma longa, Antimicrobial, S. aureus, C. albicans, E. coli, Enterococcus, K. pneumonia*

1. Introduction

Herbal medicines use plant parts for healing and disease treatment. Historically, they have been widely used, with the World Health Organization reporting that about 80% of people use them for primary healthcare. Over 35,000 plant species are utilized globally for medical purposes, some having antimicrobial, antidiabetic, antiviral, anticancer, and antifungal properties. Oral infections, such as dental caries, affect enamel and dentine and can lead to tooth loss if untreated. Imbalance in the mouth's normal flora, typically non-pathogenic bacteria, can cause infections and tooth decay (Shukla & Kumari, 2019).

Polyherbal mouthwash means that two or more plants have beneficial effects towards oral hygiene. Each plant used in the formulation of herbal mouthwash has been used traditionally value for centuries as a natural remedy for oral care in many cultures. In many societies cultural use of Neem (*Azadirachta indica*), Barro (*Terminalia bellirica),* Betel plants (*Piper betle*) and Turmeric (Curcuma longa) these plants are rich in antibacterial agent, anti-inflammatory agents, anti-oxidant and mouth freshener properties.

Periodontal diseases can destroy ligaments, cementum, gingiva, and alveolar bone. Plaque causes gingival inflammation, and its control can be achieved with instant herbal mouthwashes. These mouthwashes deliver therapeutic ingredients to combat oral organisms. Chlorhexidine (0.1%-0.2%) is a gold standard but has notable side effects, including tooth staining, contact dermatitis, and IgE-mediated hypersensitivity (Singgih et al., n.d.).

Junk foods significantly impact oral health, particularly candies, chocolates, jellies, and jams, which have high sugar content. These sugars contain insoluble glucan that adheres to tooth enamel, leading to cavities. Carbonated drinks also damage enamel, causing erosion, potential dentine exposure, and tooth discoloration. Mouthwashes are recommended to quickly remove food particles (Banu & Gayathri, 2016).

Herbal mouthwash is in high demand for its quick pain relief, effectiveness against oral pathogens, and fewer side effects. Chemical mouthwashes with hydrogen peroxide and chlorhexidine whiten, sterilize, and relieve pain but may discolor teeth

and cause side effects, despite being economical. Chlorhexidine is not ideal for long-term use due to staining, but this can be managed with proper oral hygiene. Herbal mouthwashes are suitable for prolonged use to address various dental issues (Namdeo et al., n.d.).

Azadirachta indica (Neem) has been extensively used in Ayurveda, Unani and homoeopathic medicine and has become a wonder tree of modern medicine. It has been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental problems. *Neem* twigs are used as oral deodorant, toothache reliever and for cleaning teeth. *Neem* bark possesses antibacterial and deodorant activity (Vinod et al., 2018).

The phytochemical constituents present in *neem* are nimbidin, nimbin, nimbolide, Azadirachtin, gallic acid, epicatechin, catechin, and margolone which exhibit potent antibacterial activity. The chief active constituent of *neem* is azadirachtin, which is an effective antimicrobial agent (Chaudhary et al., 2023).

Ethanolic and aqueous extract of *Neem* leaf showed significant anti-candidial effects against *C. albicans* (Kumar et al., 2022). A clinical study demonstrated the effects of the leaf aqueous extract from *Azadirachta indica* (*Neem*) on adhesion, cell surface hydrophobicity and biofilm formation, which may affect the colonization by *Candida albicans*. The results suggest that *Neem* leaves have a potential anti-adhesive effect on the sample studied *in vitro* (Hoque et al., 2012).

The Piper betel plant (Piper betle Linn.) is a native plant of Southeast Asia. Leaves of the piper betel plant contain several active compounds such as eugenol and its isomers, chavibetol, hydroxychavicol, pentatriacontanol, piperol, piperbetol, Hydroxychavicol has been examined as an antimicrobial ingredient, and it is promising for several applications. The possibility of using hydroxychavicol was evaluated from piper betel as an oral care agent and found that its antimicrobial profiles are well suited as an active ingredient for oral care products (Das & De, 2011).

Antifungal activities of hydroxychavicol from *Piper betel* extract demonstrated fungicidal effects against all the fungal species tested including *Candida* species, *Aspergillus* species, and dermatophytes including *Trichophyton rubrum* (Khosla et al., 2000).

Terminalia bellirica (Barro) is a medicinal plant which has a wide range of pharmacological activity. The major ingredients of *T. bellirica* are ellagic and gallic acid and acid derivatives including epigallocatechin gallate. Tannic acid is found to be the major constituent of the ripe fruit (Athavale, 1999). They have large phenolic groups that provide them with unique binding properties causing them to bind to

mucosal and tooth surfaces and this results in the prolonged action of the extract (Khosla et al., 2000).

The ethnobotanical use of *T. bellirica,* the fruit decoction is used as a gargle in oral ulcers and sore throats. Its powder is a good astringent dentifrice in loose gums, bleeding and ulceration in gums.

The aim is to prepare Anti-microbial polyherbal Mouthwash from the hydroalcoholic extracts of 4 different leaves namely *Azadirachta indica*(Neem), leaves of *Piper betle* (Betel plant), the fruit of *Terminalia bellirica* (Barro) and rhizomes of *Curcuma longa* (Turmeric) that acts against the common oral pathogens- *Staphylococcus aureus, Candida albicans, Enterococcus spp, Klebsiella pneumoniae and Escherchia coli* and to evaluate the Anti-microbial activity by using Agar well diffusion method.

2. Materials and Method

2.1 Test Organisms

Test organisms used in this study were collected from the pathology lab of Star Hospital, Sanepa-2, Lalitpur, Nepal. The organisms included *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*(both resistant and sensitive strains), *Enterococcus faecalis*, and *Klebsiella pneumoniae*.

2.2 Collection of Plants

The plant materials were collected from various locations in Nepal. The leaves of Neem (*Azadirachta indica*) and the Betel plant (*Piper betel*) were obtained from Lahan Municipality-9, Siraha, Nepal, on 26th Ashadh 2080 (Nepali calendar). The fruits of Barro (*Terminalia bellirica*) and the rhizome of Turmeric (*Curcuma longa*) were acquired from Ram Pharma Supplier. These plant parts were carefully selected to ensure freshness and authenticity for the study

2.3 Preparation of Plant Extract

The collected plant materials were washed with sterile water and then collected plant materials were shadow-dried at room temperature for 3-5 days. The dried plant materials were pulverized and stored in air-tight containers separately. The hydro-alcoholic solution was prepared by mixing 70% v/v of ethyl alcohol and water. Then powder of each ingredient was added to the hydroalcoholic solution in a ratio of 1:5 (Pankaj et al., 2011).

Each plant material was prepared by soaking the powdered plant parts in a prepared solution and macerating them at room temperature for 72 hours. The macerated solution was first filtered by sterilized muslin cloths for coarse residue and filtered

using sterilized Whatmann filter paper (no.1), Whatman filter paper was sterilized by using an autoclave at 121^0C and 15 psi for 30 min and measured and kept in an airtight amber-coloured container (Biswas et al., 2002; Pankaj et al., 2011). Marc was washed with 10 ml of distilled water and pressed. After being concentrated by evaporation by using a water bath at 50°C for complete drying of extract and then the powder was collected and stored in an airtight container

2.4 Formulation of Herbal mouthwash

Formulation of polyherbal mouthwash was prepared by using the following procedure as shown in Fig. 1 (Banu & Gayathri, 2016)

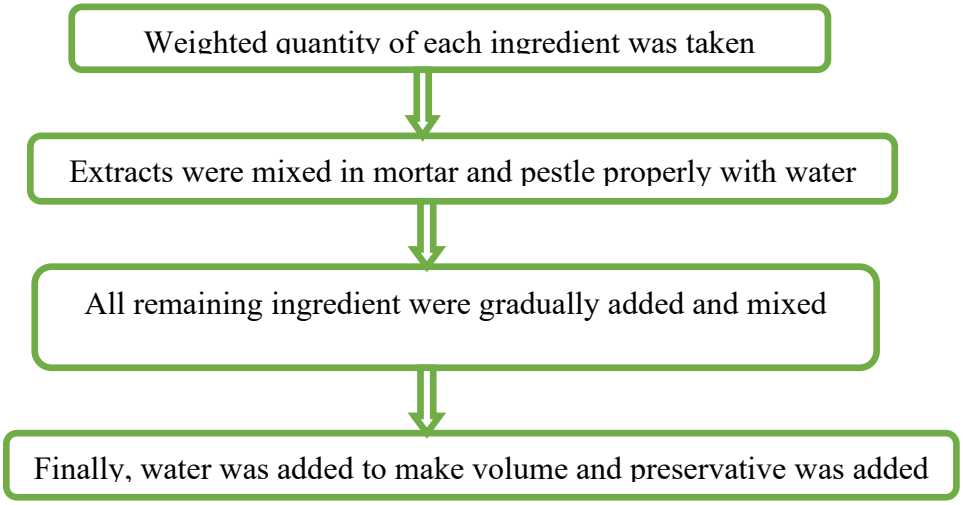

Figure 1: Flowchart of the polyherbal Mouthwash Formulation

Table 1: Formulation of Herbal Mouthwash

SN.	Ingredients	Botanical name/ Company name	Plant parts	Function	Formulation in four different quantities			
					F1	F2	F3	F4
1.	Neem	*Azadirachta indica*	Leaves	Antimicrobial	100 mg	200 mg	400 mg	800 mg
2.	Barro	*Terminalia bellirica*	Pericarp	Antimicrobial	100 mg	200 mg	400 mg	800 mg
3.	Betel plant	*Piper betle*	Leaves	Antimicrobial	100 mg	200 mg	400 mg	800 mg

4.	Turmeric	*Curcuma longa*	Rhizome	Antimicrobial	100 mg	200 mg	400 mg	800 mg
5.	Clove oil	*Eugenia caryophyllus*	Flower bud	Flavor, Analgesic	0.1 ml	0.15 ml	0.20 ml	0.25 ml
6.	Saccharine	CDH(030034)	N/A	Sweetener	0.1 mg	0.1 mg	0.1 mg	0.1 mg
7.	PEG 40	HIMEDIA	N/A	Surfactant	6 g	6 g	6 g	6 g
8.	Glycerol	HIMEDIA (GRM 081)	N/A	Co-surfactant	6.5 ml	6.5 ml	6.5 ml	6.5 ml
9.	Ethyl alcohol	CHFC Co. Ltd	N/A	Preservative	2 ml	2 ml	2 ml	2 ml
10.	Distilled water	MARECH Pvt. Ltd.	N/A	Solvent	q.s.	q.s.	q.s.	q.s.

Table No. 1 F_1, F_2, F_3, and F_4 represent formulations containing 100 mg, 200 mg, 400 mg and 800 mg extract respectively (Abbreviation: q.s. = quantity sufficient, PEG = Poly Ethylene Glycol)

2.5 Evaluation of Prepared Herbal Mouthwash

2.5.1 Physical Evaluation

Colour, Odour and Consistency were examined by using the visual examination of prepared herbal mouthwash.

Colour: Light- Orange

Odour: pleasant aromatic odour

Consistency: homogenous solution

a. pH: A digital pH meter was used to measure the pH of the prepared herbal mouthwash and careful reading should be taken and recorded in the notebook. It was repeated for three times and the average reading should be calculated properly. The average calculated pH of the prepared herbal mouthwash was slightly acidic in nature (Sunitha et al., 2009).

b. Relative Density: It is the ratio of the density of the substance to the density of pure water. it is also known as the specific gravity. the density of water is essential at room temperature. Thus, the relative density of the substance is very nearly equal to its density and it has no unit. Density was measured by

using a pycnometer which represents mass per unit volume in the unit's g/ml or g/cm^3. Firstly, we were washed the pycnometer and dried it in the hot air oven for complete drying of the apparatus and then left it for cooling and then only used it for the measurement of the density of the water and prepared herbal mouthwash (Basir et al., 2023; Viana, 2002).

Relative

$$\text{density}(\rho) = \frac{\text{Mass of liquid}(W3-W1)}{\text{Mass of equal volume of water}(W2-W1)} \quad \text{Eq 1}$$

c. Viscosity: Viscosity was measured by using Ostwald's viscometer, Firstly water was passed through it and time was recorded and reading was recorded in the notebook. After that prepared herbal mouthwash was passed through the same Ostwald viscometer, which was repeated three times and their average time should be calculated carefully.

According to Ostwald's viscosity of the sample could be calculated as the following formula (Beaulieu et al., 2017).

$$\text{Viscosity sample } (\eta_2) = \frac{\rho 2 * t2}{\rho 1 * t1} * \eta_1 \quad \text{Eq 2}$$

Where,

η_1=Viscosity of water

η_2=Viscosity of sample

ρ_1=Relative density of distilled water

ρ_2=Relative density of the sample

t_1=Time of water pass A to B mark of Ostwald Viscometer.

t_2=Time of the sample pass A to B mark of Ostwald Viscometer.

2.5.2 Preparation of Agar Media

Mueller Hinton Agar (MHA) was used to prepare the agar media to provide the nutrition for the growth of bacteria. It was prepared by using sterile distilled water in the conical flask, all the required apparatus was washed properly and dried in the hot air oven. Suspend 38 gm of the medium in one-litre distilled water and the prepared solution was heated with frequent stirring and boiled for one minute to completely dissolve the medium. The prepared MHA solution was sterilized in the autoclave at 121^{o}C and pressure was adjusted to 15lb and sterilized for 15 minutes, the solution was left to cool the solution at room temperature. A sterilized petri plate was taken. The prepared solution of MHA was poured into the plate inside the laminar airflow to

maintain the aseptic technique and was left to solidify the prepared media completely and the prepared plate was stored in the refrigerator by adjusting the temperature between 2^0C - 8^0C (Shanthi & Radha, 2020).

2.5.3 Antibacterial Sensitivity Test (Agar Well Diffusion Method)

An antibacterial activity test was performed on the isolated colonies of bacteria which were taken from the microbiology lab of the Star Hospital. The agar well diffusion method was used to determine the zone of inhibition. The prepared MHA media agar plate was taken and an aseptic environment was created by burning of burner at four different corners of the working table, The working area should be free of microbes. The isolated bacteria were handled carefully and the code of the bacteria should be noted properly. After being created in an aseptic area bacteria were inoculated on the Petri plate by using the streaking method to distribute the bacteria equally in the plate, After the completion of the distribution of bacteria, a well was formed at six different places in the plate carefully calculating the distance between each well, by using the agar well cutter the well was formed properly and then the well was loaded 100µl with the help of micropipette of prepared herbal mouthwash which was prepared in the different concentration F_1, F_2, F_3, F_4, blank and standard at the coded well of plate respectively. After the completion of loading of the well, the plate was left 1 hour undisturbed for the passive diffusion of the herbal mouthwash into the culture media. The loaded culture media plate was incubated at 37ºC for 24 hours and a triplicate method was used & The experiment was repeated three times and the zone of inhibition was measured & and calculated, noted in the notebook careful (Namdeo et al., n.d.).

2.5.4 Statistical Data Analysis

The experiments were repeated three times and the values were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA using IBM SPSS Statistics version 29.0.1.0(171) and a significance value of $P<0.05$.

3. Result

The pH of the different formulations is slightly acidic F_1, F_2, F_3, and F_4 are 5.6, 5.6, 5.7, and 5.7 as compared to Chlorhexidine mouthwash pH was 5.9 as shown below in Table 2. Hence, the pH values for different formulations (F_1, F_2, F_3, and F_4) were slightly acidic, ranging from 5.6 to 5.7. In comparison, the Chlorhexidine mouthwash had a pH of 5.9. The results suggest that the prepared mouthwashes are within the suitable pH range for oral use

3.1 Evaluation of relative density of different formulations

The evaluation of the relative density of different quantities of formulation is represented in sample Blank, F_1, F_2, F_3, F_4 and Chlorhexidine. Where W_1 represent the weight of an empty specific gravity Pycnometer, W_2 represent the weight of a specific gravity Pycnometer + Distilled water and W_3 represent the weight of a specific gravity Pycnometer + Sample. According to the method, relative density of quantities formulation were measured and noted in the table as shown below in Table No.3. The relative density of the formulations (F_1, F_2, F_3, F_4) and Chlorhexidine ranged around 1.03 to 1.04, indicating that the formulations are slightly denser than water. This density is considered appropriate for oral formulations

Table 2: Result and Evaluation of pH of different formulation

S.N	Sample	pH_1	pH_2	pH_3	Mean pH
1.	Blank	5.4	5.7	5.5	5.5±0.12
2.	F_1	5.4	5.6	5.8	5.6±0.16
3.	F_2	5.9	5.6	5.5	5.6±0.18
4.	F_3	5.8	5.6	5.9	5.7±0.08
5.	F_4	5.6	5.9	5.7	5.7±0.12
6.	Chlorhexidine	6.1	5.8	5.9	5.9±0.12

The evaluation result of pH is expressed in the mean ± standard deviation.

Table 3: Evaluation of relative density of different formulations

S.N	Sample	W_1(gm)	W_2 (gm)	W_2-W_1	W_3 (gm)	W_3-W_1	Relative Density(ρ)
1.	Blank	26.74	80.47	53.73	82.37	55.63	1.03
2.	F_1	28.05	77.73	49.68	79.93	51.88	1.04
3.	F_2	30.15	81.52	51.37	83.04	53.09	1.03
4.	F_3	26.27	71.69	53.42	81.82	55.55	1.03
5.	F_4	29.77	79.82	50.05	81.67	51.90	1.03
6.	Chlorhexidine	27.57	81.43	53.86	82.64	55.07	1.02

3.2 Evaluation of Viscosity of the different Formulations

The viscosity of different formulations were measured and noted as shown below in Table no. 4. Where T_1, T_2, and T_3 represent the times to repeat the experiment and the average value of these values were calculated.

Table 4: Evaluation of viscosity of the different formulations

S.N	Sample	Time (sec)			Mean Time (sec)	Relative Density of Sample ρ(g/cc)	Viscosity(η)in mPa.
		T_1	T_2	T_3			
1.	Water	1.34	1.32	1.35	1.33	1.00	1.00
2.	Blank	1.52	1.42	1.43	1.45	1.03	1.12
3.	F_1	1.42	1.45	1.41	1.42	1.04	1.11
4.	F_2	1.43	1.39	1.45	1.42	1.03	1.09
5.	F_3	1.42	1.41	1.46	1.43	1.03	1.10
6.	F_4	1.43	1.45	1.47	1.45	1.03	1.12
7.	Chlorhexidine	1.23	1.43	1.39	1.35	1.02	1.03

3.3 Result of Agar Well Diffusion Antimicrobial Test of F_1

The evaluation of the agar well diffusion antimicrobial test of formulation F_1 for isolated micro-organism are shown below Table No.5 . Where X_1, X_2, and X_3 represent the repeated times of average triplicate data respectively

Table 5: Result of Agar Well Diffusion Antimicrobial Test of formulation F_1

SN.	Organism	Zone of Inhibition (mm)				
		Blank	F_1			Mean ± Standard Deviation
			X_1	X_2	X_3	
1.	*C. albicans*	0	18	19	21	19.00±1.24
2.	*S. aureus*	0	12	11	14	12.34±1.24
5.	*E. feacalis*	0	13	15	11	13.00±1.6
3.	*E. coli* (resistant)	0	0	0	0	0±0
4.	*E.coli* (sensitive)	0	0	0	0	0±0

6.	*Klebsiella pneumoniae*	0	0	0	0	0±0

The zone of inhibition of F_1 on growth agar media expressed in mean ± standard deviation.Where, X_1, X_2 and X_3 are respective times to represent the experiment respectively.

Table 6: Result of Agar Well Diffusion Antimicrobial Test of F_2

SN.	**Organism**	**Zone of Inhibition (mm)**				
		Blank	**F_2**			**Mean ± Standard Deviation**
			X_1	**X_2**	**X_3**	
1.	*C. albicans*	0	21	23	19	21.00±1.6
2.	*S. aureus*	0	14	17	15	15.34±1.24
3.	*Enterococcus*	0	13	14	17	14.67±1.69
4.	*E. coli* (resistant)	0	0	0	0	0
5.	*E.coli* (sensitive)	0	0	0	0	0±0
6.	*Klebsiella pneumoniae*	0	0	0	0	0±0

Where, X_1, X_2 and X_3 are respective times to represent the experiment respectively. Note: The zone of inhibition of F_2 on growth agar media expressed in mean and standard deviation

Table 7: Result of Agar Well Diffusion Antimicrobial Test of F_3

SN.	**Organism**	**Zone of Inhibition (mm)**				
		Blank	**F_3**			**Mean ± Standard Deviation**
			X_1	**X_2**	**X_3**	
1.	*C. albicans*	0	22	25	27	24.67±2.04
2.	*S. aureus*	0	18	15	17	16.66±1.24
3.	*Enterococcus*	0	21	19	23	21.00±1.66
4.	*E. coli* (resistant)	0	0	0	0	0±0
5.	*E.coli* (sensitive)	0	0	0	0	0±0
6.	*Klebsiella pneumoniae*	0	0	0	0	0±0

Note: The zone of inhibition of F_2 on growth agar media. Where, X_1, X_2 and X_3 are respective times to represent the experiment respectively.

The zone of inhibition of F_3 on growth agar media expressed in mean ± standard deviation.

Table 8: Result of Agar Well Diffusion Antimicrobial Test of F_4

S.N	Organism	Zone of Inhibition (mm)				
		Blank	**F_4**			**Mean ± Standard Deviation**
			X_1	**X_2**	**X_3**	
1.	*C. albicans*	0	27	24	28	26.33±1.63
2.	*S. aureus*	0	22	19	21	20.66±1.23
3.	*Enterococcus*	0	22	20	25	21.66±2.35
4.	*E. coli* (resistant)	0	0	0	0	0±0
5.	*E.coli* (sensitive)	0	0	0	0	0±0
6.	*Klebsiella pneumoniae*	0	0	0	0	0±0

Note: Where, X_1, X_2 and X_3 are respective times to represent the experiment respectively.

Table 9: Result of Agar Well Diffusion Antimicrobial Test of Standard

SN.	**Organism**	**Zone of Inhibition (mm)**				
		Blank	**Standard (Chlorhexidine)**			**Mean ± Standard Deviation**
			X_1	**X_2**	**X_3**	
1.	*C. albicans*	0	27	24	26	25.66±1.24
2.	*S. aureus*	0	21	22	19	20.66±1.24
3.	*Enterococcus*	0	19	23	21	21.00±1.6
4.	*E. coli* (resistant)	0	17	20	19	18.66±1.49
5.	*E.coli* (sensitive)	0	15	18	17	16.66±1.24

6.	*Klebsiella pneumoniae*	0	13	15	16	14.66±1.24

Where, X_1, X_2 and X_3 are respective times to represent the experiment respectively.

Table 10: Result of Agar Well Diffusion Antimicrobial Test

S.N	Organism	Zone of Inhibition (mm)					
		Blank	F_1	F_2	F_3	F_4	Standard (Chlorhexidine)
1.	*C. albicans*	0	19.00±1.24	21.00±1.6	24.67±2.04	26.33±1.63	25.66±1.24
2.	*S. aureus*	0	12.34±1.24	15.34±1.24	16.66±1.24	20.66±1.23	20.66±1.24
3.	*Enterococcus*	0	13.00±1.6	14.67±1.69	21.00±1.66	21.66±2.35	21.00±1.6
4.	*E. coli* (resistant)	0	0±0	0	0±0	0±0	18.66±1.49
5.	*E.coli* (sensitive)	0	0±0	0±0	0±0	0±0	16.66±1.24
6.	*Klebsiella pneumoniae*	0	0±0	0±0	0±0	0±0	14.66±1.24

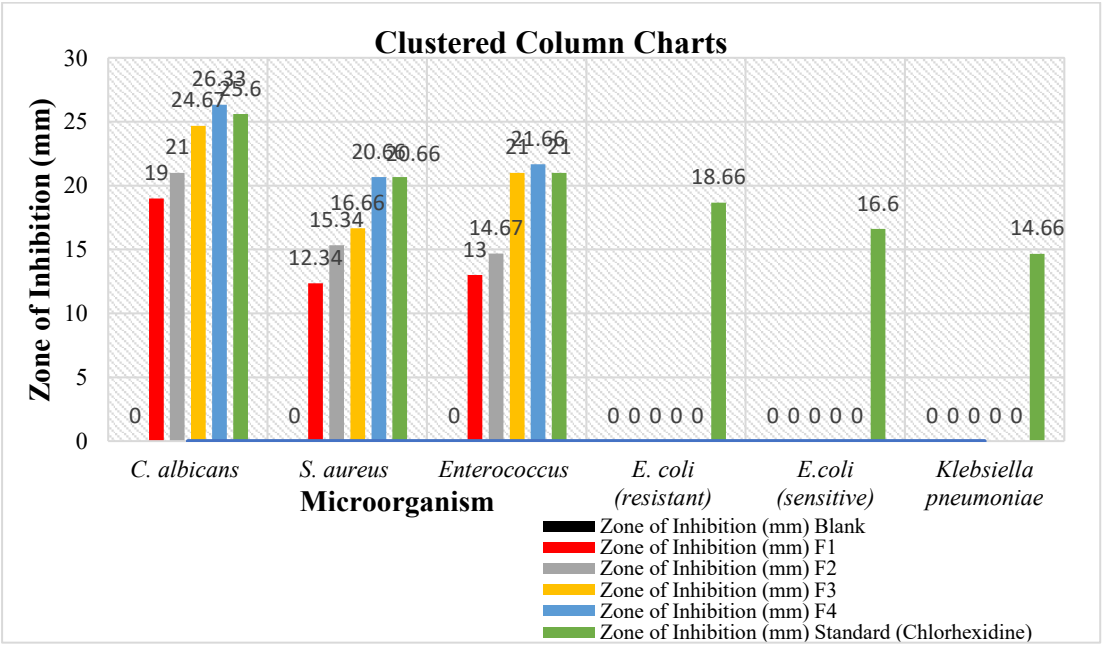

Figure 2: Result of Agar Well Diffusion Antimicrobial Test of microorganism.

The collective summary across all formulations (F1, F2, F3, F4) demonstrated varying degrees of antimicrobial activity against tested microorganisms. The standard Chlorhexidine also showed comparable effectiveness.

Table 11: Formulation showing significance with standard

Organism	Zone of Inhibition (mm)				
	F_1	**F_2**	**F_3**	**F_4**	**Chlorhexidine**
C. albicans	19.00*	21.00	24.67	26.33	25.66
S. aureus	12.34*	15.34	16.66	20.	20.66
Enterococcus	13.00*	14.67*	21.00	21.66	21.00

* The mean difference is significant at the 0.05 level as compared to the standard.

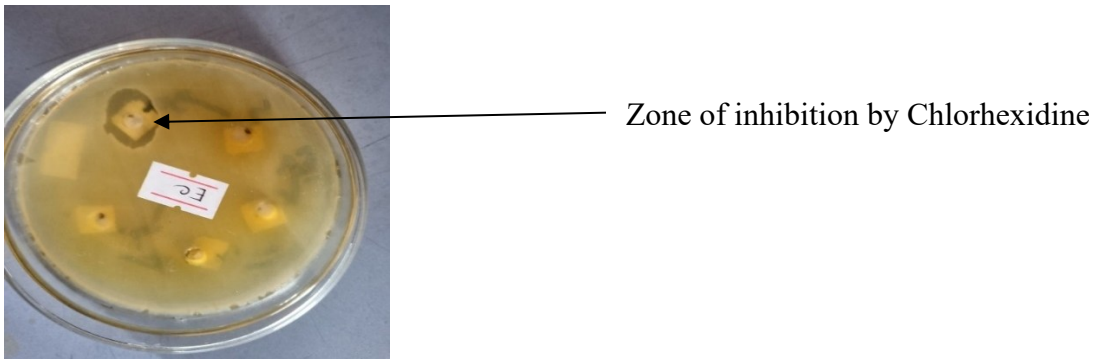

Figure 3: Zone of inhibition of *E. coli* (Sensitive)

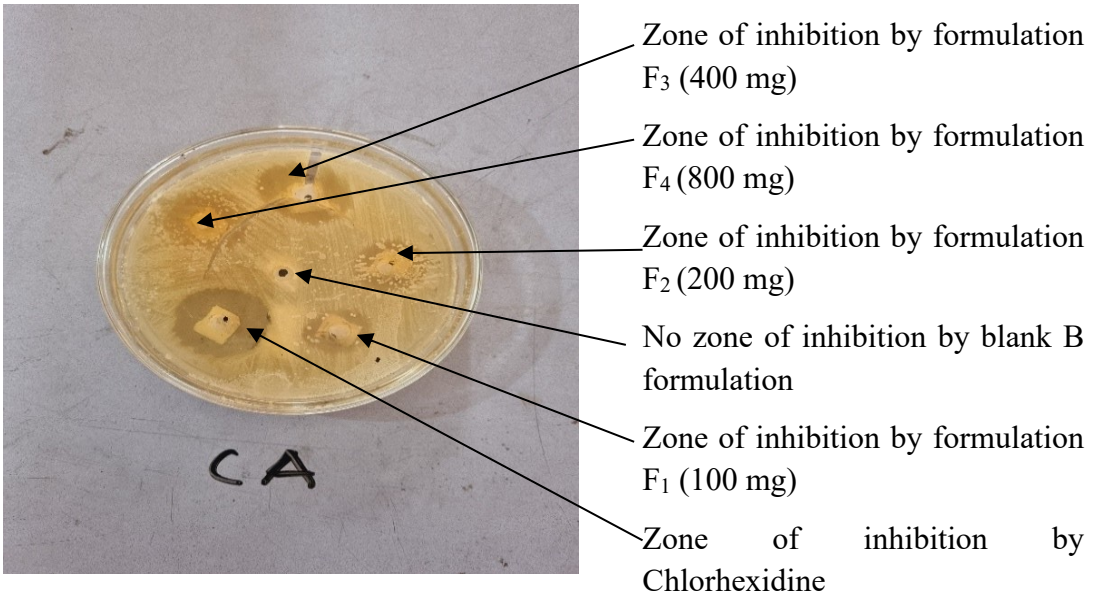

Figure 4: Zone of Inhibition of *C. albicans*

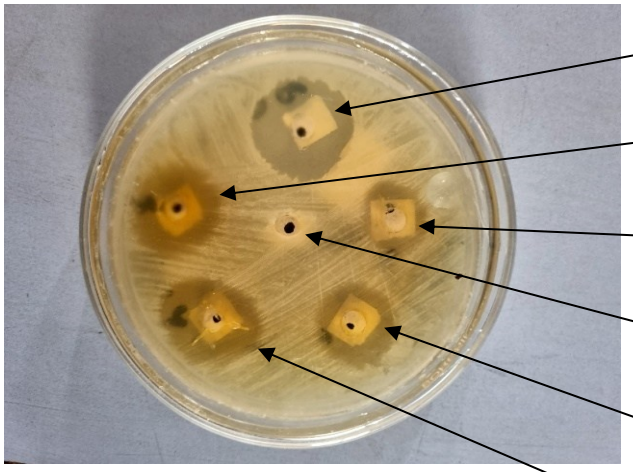

Figure 5: Zone of nhibition of *E. faecalis*

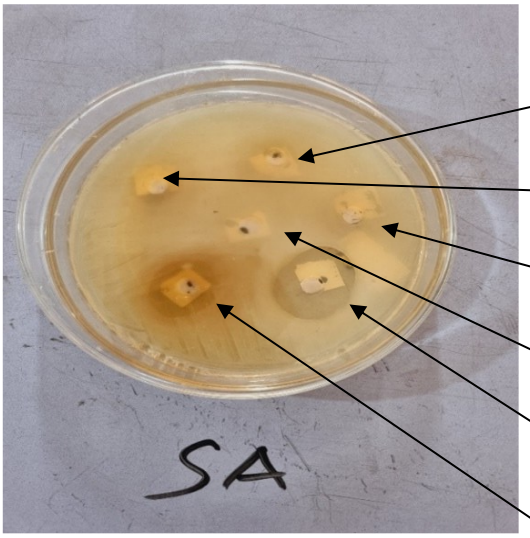

Figure 6: Zone of Inhibition of *S. aureus*

4. Discussion

Herbal mouthwash is prepared from the extract of leaves of neem, leaves of betel plant, rhizome of turmeric and pericarp of barro. The prepared herbal mouthwash is formulated in four different formulations F_1, F_2, F_3 and F_4 among them F_4 formulation is the best formulation as compared to the standard. The pH was found to be 5.7 which was found within the range of pH suitable for the mouthwash and the pH of the standard was 6.1 and the relative density & viscosity of the prepared herbal mouthwash were found to be 1.03 g/cm3 and 1.12 mPa. Sec respectively, which is easily pourable and completely poured into the container. Similarly, the relative

density and viscosity of standard mouthwash are found to be 1.02 g/cm3 and 1.03 mPa.sec respectively.

The prepared herbal mouthwash is found to be highly effective in *C. albicans, S. aureus* and *E. faecalis.* Similarly, formulated herbal mouthwash is found resistant to *E. coli* (resistant), *Klebsiella pneumoniae* and *E. coli* (sensitive). The antimicrobial efficacy of the prepared herbal mouthwash is tested by using the agar well diffusion method and by measuring their zone of inhibition of the bacteria. The zone of inhibition of prepared mouthwash in *C. albicans (*26.33mm), S. aureus (20.66mm), *E. faecalis* (21.66mm) and zone of inhibition of standard in *C. albicans* (25.66mm), *S. aureus*(20.66mm), *E. faecalis* (21.00mm), [*E.coli*(resistant) (18.66mm)], *K. pneumoniae*(14.66mm), [E.coli(sensitive) (16.66mm)].

Prepared herbal mouthwash was found sensitive to *C. albicans, S. aureus* and *Enterococcus* and found resistant to *K. pneumoniae, E. coli*(resistant) and *E. coli* (sensitive). By observing the above zone of inhibition of the bacteria, we can say that our prepared herbal mouthwash is comparatively highly effective in *C. albicans*, *S. aureus* and *E. faecalis* as compared to the standard (chlorhexidine 0.2%). The F_4 formulation is the best alternative to chlorhexidine in candidiasis and Enterococcal infection. This formulation is found resistant in gram-negative bacteria and highly effective in gram-positive bacteria.

5. Conclusion

Results obtained in this study conclude that the extract of Neem leaves, leaves of Betel plant, rhizome of Turmeric and pericarp of Barro can be used to prepare polyherbal mouthwash as the mouth has antimicrobial activity against oral pathogens. The significant antimicrobial efficacy of the herbal mouthwash in this study is due to the presence of bioactive compounds in the plant extract. Therefore, the zone of inhibitory is shown by herbal mouthwash containing the above plant extract against *C. albicans, E. faecalis and S. aureus* proving that polyherbal mouthwash can be used to replace the use of chemical mouthwash in oral hygiene. As a result, formulated polyherbal mouthwash will help in the maintenance of oral hygiene.

Limitation and Recommendation

The study on the herbal mouthwash formulated from neem leaves, betel leaves, turmeric rhizome, and barro pericarp, while promising, has several limitations. It only examines a few formulations (F1 to F4), limiting the scope for finding potentially more effective alternatives. Additionally, a dose-dependent study was not performed, which is necessary to establish the optimal concentration of each extract. The study also lacks an evaluation of interactions between different plant extracts and excipients, which could affect the mouthwash's stability and efficacy.

To address these limitations, future research should include clinical trials to confirm safety and efficacy in a broader population. Dose-dependent studies are recommended to determine the ideal extract concentration. Exploring different extraction methods, such as aqueous or combined with ethanol, and assessing toxicity would further validate the herbal mouthwash's potential benefits.

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