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Combination of Chlorhexidine with magic mouth wash for the prophylaxes of chemotherapy induced mucositis

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Abstract:

Daily chlorhexidine mouthwash is often recommended for preventing chemotherapy-induced oral mucositis. A stable mouthwash was formulated for the management of chemotherapy induced oral mucositis. There are various approaches to manage oral mucositis which involves the combination of different ingredients such as analgesics, local anesthetics and muco-protective agents. However, there is a likelihood of developing microbial colonization because of the disruption of mucosal membrane, therefore antifungal or antibacterial are added in the formulation prophylactically, which may also treat other prevailing infection, if any. A well-known product, magic mouthwash was modified with the addition of chlorhexidine and checked for its physical stability, taste and efficacy in terms of antimicrobial activity at different storage conditions.

Key Words: chlorhexidine, chemotherapy, mucositis, prophylactically, antifungal, antibacterial

Introduction

Mucositis is a common complication. Chemotherapeutic agents and / or radioactive energy used to treat cancer break down and rapidly divide into epithelial cells of the gastrointestinal tract. Mucositis can occur anywhere in the gastrointestinal tract, but the most common site is the mouth.

Approximately 20% to 40% of patients receiving conventional chemotherapy, 80% of patients receiving high-dose chemotherapy in the form of hematopoietic stem cell transplantation (HSCT) and nearly all patients receiving head and neck radiation therapy all (H&NRT). Oral mucositis (OM) manifests as erythema and / or ulcers of the oral mucosa. In addition, the mucous membranes of the pharynx, larynx, and esophagus are also at risk of developing mucositis, especially in patients undergoing H & NRT. The incidence of OM is primarily associated with pain associated with inflammation of the oral mucosa and ulcers. The pain of mucositis can adversely affect food intake, oral intake, including oral medications, maintenance of oral hygiene, and quality of life.

Like mucositis, as with all complications of cancer treatment, the goal is to prevent it from occurring. Most preventive measures focus on oral mucositis, which usually requires maintaining good oral hygiene, keeping the mucous membranes clean, and keeping them away from the source of infection. With these goals in mind, doctors may prescribe mouthwashes such as baking soda and saline, or magic mouthwashes.

There are various scales that can be used to measure oral mucositis. The most commonly used scale is the World Health Organization (WHO) and the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE).

Table 1.1: NCI Scale for Measuring Oral Mucositis

NCI Scale	
Grade 0	No
Grade 1	Painless ulcers, erythema, or mild pain in the absence of ulcers
Grade 2	Have painful erythema, swelling, or ulcers, but can be eaten or swallowed
Grade 3	Painful erythema, swelling, or ulcer that requires intravenous fluidation
Grade 4	Severe ulcers require parenteral or enteral nutrition or prophylactic intubation.
Grade 5	Toxicity-related death

Table 1.2: WHO Scale for Measuring Oral Mucositis

WHO Scale	
Grade 0	No
Grade 1	Pain +/- no erythema, no ulcer
Grade 2	Patients with erythema or ulcers can eat a solid diet.
Grade 3	Ulcers, widespread erythema, patients cannot eat solid food.
Grade 4	Oral mucositis to the extent of indigestible

Basic oral care includes brushing your teeth, good oral hygiene, and moisturizing your mouth with regular saline / bicarbonate rinses to help moisturize your oral tissue. In addition to basic oral care during cancer treatment, the

following therapeutic interventions are recommended based on well-designed studies of anti-inflammatory, antibacterial, analgesic, and complex mouthwashes.

Several studies have evaluated a variety of topical agents, including topical analgesics, to treat the pain of mucositis. Another problem with aphthous ulcerates is related to the colonization of oral ulcers by the microflora. Although mucositis is not the cause of infection, secondary microbial colonization of oral lesions can cause clinically significant local or systemic infections and theoretically exacerbate the severity of mucositis. Therefore, the effectiveness of antibiotics on OM was also evaluated. Formulated products such as "magic mouthwash" are usually coatings with various ingredients added. They usually contain local anesthetics, sometimes magnesia milk-based antihistamines, antifungals, and preservatives. Products are usually mixed in equal percentages of the mixture of each component, thereby diluting each component in the rinse solution and reducing the concentration of each drug in the rinse solution. Applying a local anesthetic to the surface of an ulcer can cause burns in the mouth, discomfort the taste, and interfere with the pharyngeal reflex. Even if it works, it will recover in 5 to 20 minutes. Therefore, they can be consumed before meals, but paradoxically, they can reduce taste and mouthfeel, thereby reducing oral food intake. Accordingly, these compounded rinse products are not recommended by MASCC/ISOO.

Use of mouth wash will be beneficial for the patients undergoing chemotherapy, as mucositis may worsen the oral intake of the medication and it will be easy for the patient to use. Chlorhexidine and magic mouth wash are most commonly employed. However if two separately management goals are combined, cost effectiveness and the patient compliance can be enhanced.

Materials and Methods

Materials

1. Dexamethasone
2. Promethazine HCl
3. Aluminum Hydroxide
4. Nystatin
5. Lignocaine HCl
6. Chlorhexidine Gluconate
7. Water

Preparation

Preparation of Magic Mouthwash

The ingredients used for the formulation of magic mouthwash are shown in the table 2.1 along with their strength, quantity used and role.

Table 2.1: Role of Ingredients

S.No	Ingredient	Strength	Quantity	Manufacturer	Role of Ingredient

1	Dexamethasone	0.5mg	20 tablets	Progressive Laboratories	It is a corticosteroid which reduces swelling and inflammation
2	Promethazine	25mg/5ml	120ml	Sonafi Aventis (Pakistan) Ltd	It is used for the prevention of allergic reactions.
3	Aluminum Hydroxide and Magnesium Hydroxide	291mg/5ml	120ml	Pfizer Laboratories Ltd.	Aluminum hydroxide is an antacid.
4	Nystatin	100000IU/ml	30ml	Pfizer Laboratories Ltd.	It acts as a fungistatic and fungicidal.
5	Lignocaine	4%w/v	50ml	Barrett Hodgson Pakistan (Pvt) Ltd	It is used as a local anesthetic.
6	Chlorhexidine Gluconate	0.2%w/v	60ml	Platinum Pharmaceuticals Pvt Ltd	Chlorhexidine is antibacterial agent and topical disinfectant

The standard method used for the preparation of magic mouthwash employs the step-by-step mixing as follows:

1. Triturate 20 tablets of 0.5mg dexamethasone to a very fine powder.
2. Add about 60ml of the promethazine syrup to make paste.
3. Add 120ml Aluminum Hydroxide and Magnesium Hydroxide suspension to above paste.
4. Rinse the Aluminum hydroxide bottle with the left over promethazine and add it into the suspension formed.
5. Add 30 ml nystatin drop wise with continuous stirring.
6. Add 50 ml lignocaine followed by 60 ml chlorhexidine gluconate.
7. Finally make up the volume with water to 480ml (8 bottles each of 60 ml).

Tests

Stability

Stability of the magic mouth wash checked by analyzing the physical properties such as appearance, odor, taste and sedimentation of the freshly prepared formulation were checked of the preparation stored at room temperature (25^oC) and as well in a refrigerator (2^oC-8^oC).

Microbial Testing

The formulation stored at room temperature and refrigerator was checked for anti-bacterial and anti-fungal activity by ditch plate method. Readings were noted on day 7, day 14 and day 21 using the following procedure.

Preparation of Media

Nutrient Agar medium

Beef Extract	3.0g
Peptone	5.0g
Agar	15.0g
NaCl	4.0g
Water	1000.0ml

pH adjusted to neutral (6.8) at 25 °C.

1. Dissolve the dehydrated medium in an appropriate amount of distilled water. That is, dissolve 28 g of dehydrated nutrient agar medium in 1000 ml of distilled water.
2. Stir frequently and heat for 1 minute to bring to a boil to completely dissolve the powder.
3. Sterilize the medium in an autoclave (at 121 ° C for 15 minutes).
4. Dispense the medium into tubes or plates. Place and store the agar medium.
5. Using a pH meter, determine the pH of the medium (pH 6.8 +/- 0.2) and adjust if necessary.

Preparation of Dilutions

- Original: 10 ml of preparation
- 1^{st} dilution: 1ml of original+ 9ml water (labeled as $1/10^{th}$)
- 2^{nd} dilution: 1ml of 1^{st} dilution+ 9ml water (labeled as $1/100^{th}$)
- Same procedure was carried out for preparations stored in fridge as well as at room temperature.

Preparation of Cultures

1. A loop full of a bacterial sample (Micrococcus lutea) was dissolved in 10ml of water by shaking.
2. A loop full of a sample of fungus was dissolved in 10ml of water by shaking.

Ditch Plate Method

Take 5 sterilized petri dishes, where one of the petri dishes acts as control.

For Anti-Bacterial Activity

1. Take two petri dishes; label them as anti-bacterial fridge and anti-bacterial room temperature respectively.
2. Add 1ml of the bacterial suspension, followed by the addition of the agar media to both petri dishes.
3. Shake them gently and allow them to solidify.
4. Punch the solid agar plates with wells having a diameter of 7 mm using a bore.
5. In the anti-bacterial fridge, fill the wells with 1ml of the original preparation, $1/10^{th}$ dilution and $1/100^{th}$ dilution of fridge stored preparation.
6. In the anti-bacterial bacterial, fill the wells with 1ml of the original preparation, $1/10^{th}$ dilution and $1/100^{th}$ dilution of preparation stored at room temperature.
7. Label them properly.
8. Incubate for 7 days.

For Anti-Fungal Activity

1. Take two petri dishes; label them as anti-fungal fridge and anti-fungal room temperature respectively.
2. Add 1ml of the fungal suspension, followed by the addition of the agar media to both petri dishes.
3. Shake them gently and allow them to solidify.
4. Punch the solid agar plates with wells having a diameter of 7 mm using a bore.

5. In the anti-fungal fridge, fill the wells with 1ml of the original preparation, $1/10^{th}$ dilution and $1/100^{th}$ dilution of fridge stored preparation.
6. In the anti-fungal fungal, fill the wells with 1ml of the original preparation, $1/10^{th}$ dilution and $1/100^{th}$ dilution of preparation stored at room temperature.
7. Label them properly.
8. Incubate for 14 days.

After incubation, zones of inhibition are observed in the 4 petri dishes. They were measured using a Vernier caliper, in millimeter. Then four readings and mean was calculated.

Results

Magic mouthwash is prepared by mixing dexamethasone, promethazine, aluminum hydroxide with magnesium hydroxide, nystatin, linocaine, and chlorhexidine gluconate. For the treatment of oral mucositis by chemotherapy. It is known for its anti-bacterial and anti-fungal properties. Its stability by keeping in view the appearance odor, taste and sedimentation were evaluated. Its microbial study was also studied over the passage of time.

Stability

It is a turquoise colored suspension, with a fluoride like odor and tolerable, pleasant taste. The magic mouthwash prepared sediments on standing but reconstitutes uniformly upon shaking.

Microbiological Activity

Day 1

Table 3.1- Day 1-Room Temperature

	Anti-Bacterial			Anti-Fungal		
	Original	$1/10^{th}$	1/100th	Original	1/10th	$1/100^{th}$
Mean	26.4	25.0	0	16.2	17.0	41.0
	36.55	21.7	0	17.1	23.7	33.1
	29.4	20.45	0		19.4	43.3
	31.3	14.2	0	15.4	20.0	34.1
	30.91	20.33	0	16.25	20.0	37.88

Fig 3.0 and Fig 3.1 shows that at room temperature i.e. at 25^0C, on diluting the preparation the anti-bacterial activity also decreases, whereas the anti-fungal activity increases with the decreasing concentration.

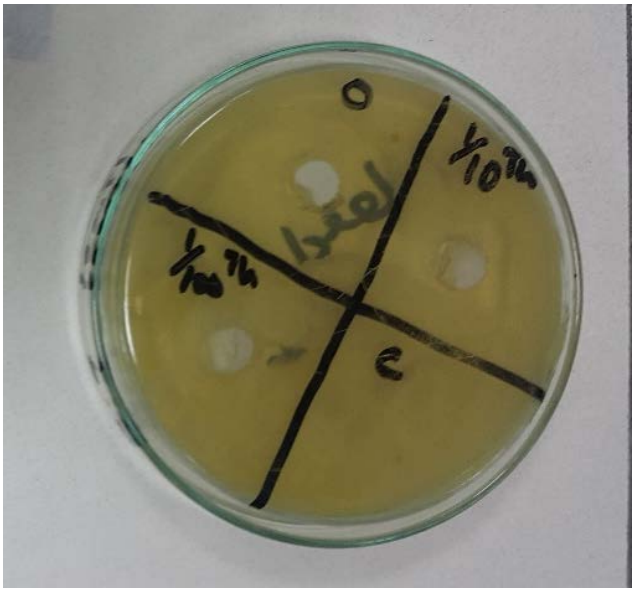

Fig 3.0: Anti-Bacterial Activity on Day 1 at 25^{o}C

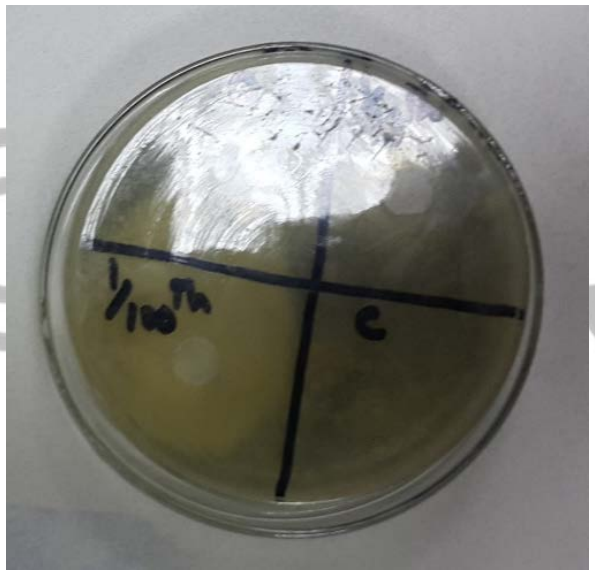

Fig 3.1: Anti-Fungal Activity on Day 1 at 25^{o}C

Table 3.2: Day 1-Fridge Temperature

	Anti-Bacterial			**Anti-Fungal**		
	Original	1/10th	1/100th	Original	1/10th	1/100th
Mean	31.0	13.9	15.5	18.5	21.4	21.8
	34.6	19.7	15.1	25.1	19.2	27.6
	24.5	23.6	0	20.9	22.2	21.1
	26.1	20.5	0	16.0	17.5	31.1
	29.05	20.18	15.3	20.125	20.1	25.4

Table 3.2 shows that the antibacterial activity becomes half at 1\100th of that it was original whereas the anti-fungal action is maximum for 1/100th dilution and minimum for the original preparation. This is showed by the zone of inhibition in fig 3.2 and Fig 3.3.

Fig 3.2: Anti-Bacterial Activity on Day 1 at 2^{o}C-8^{o}C

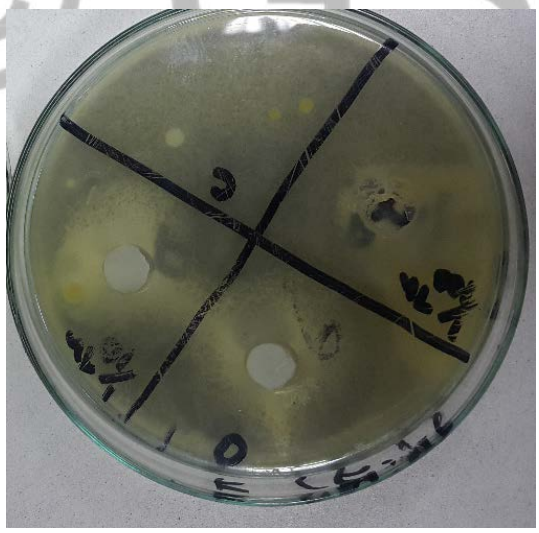

Fig 3.3: Anti-Fungal Activity on Day 1 at 2^{o}C-8^{o}C

Day 7

Table 3.3: Day 7-Room Temperature

	Anti-Bacterial			**Anti-Fungal**		
Mean	Original	1/10th	1/100th	Original	1/10th	1/100th
	19.2	17.5	0	19.0	0	0
	16.1	12.2	0	15.4	0	0
	16.4	14.0	0	14.6	0	0
	18.5	13.4	0	14.1	0	0
	17.55	14.275	0	15.775	0	0

Table 3.3 shows a minor decrease in anti-bacterial activity in the original and 1\10th dilution and a loss in activity in 1\100th dilution. The zone of inhibition is only visisble in the ditch with the original preaparation. There is no anti-fungal activity in the ditches with dilution at 25^{o}C. On day 7, both antibacterial and antifungal properties decreased as the concentration decreased.

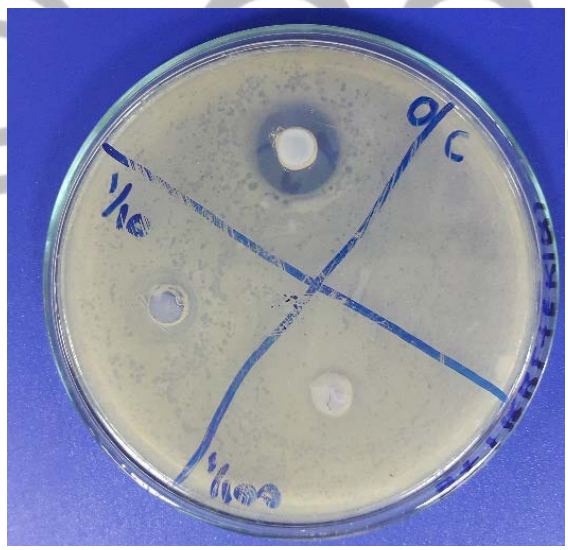

Fig 3.4: Anti-BacterialActivity on Day 7 at 25^{o}C

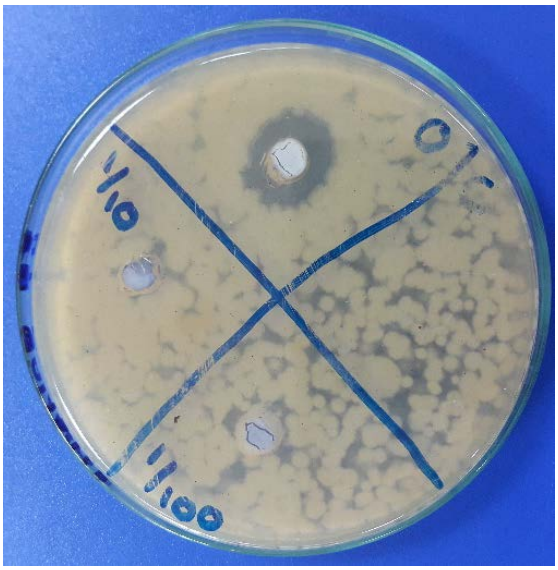

Fig 3.5:Anti-FungalActivity on Day 7 at 25^{o}C

Day 7-Fridge Temperature

	Anti-Bacterial			**Anti-Fungal**		
	Original	1/10th	1/100th	Original	1/10th	1/100th
Mean	16.6	10.7	0	20.1	13.4	10.7
	15.5	9.6	0	17.5	16.3	9.1
	16.8	10.2	0	18.1	15.6	9.2
	14.1	13.4	0	17.7	14.1	9.9
	15.75	10.975	0	18.35	14.875	9.725

Both anti-bacterial and anti-fungal activities both show a similar pattern i.e. decrease in with the decreasing concentration. The zone of inhibitions for original preparation is 15.7mm, 1\10th is 10.975mm and at 1\100th it is completely absent.

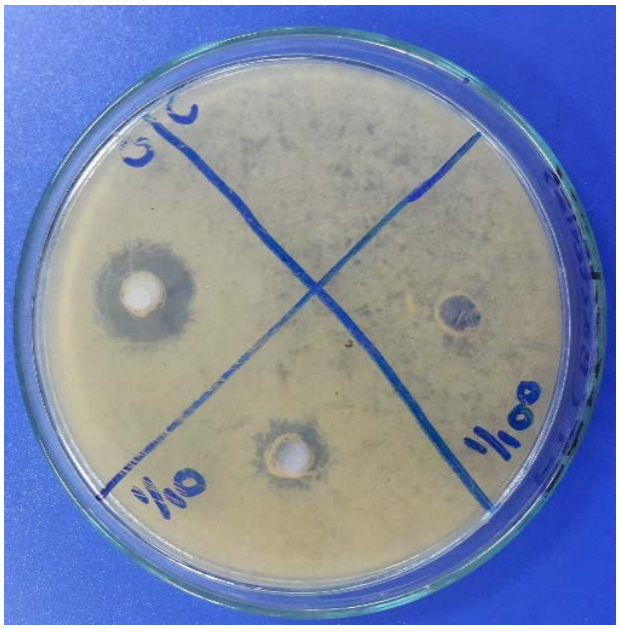

Fig 3.6 Anti-BacterialActivity on Day 7at 2^{o}C-8^{o}C

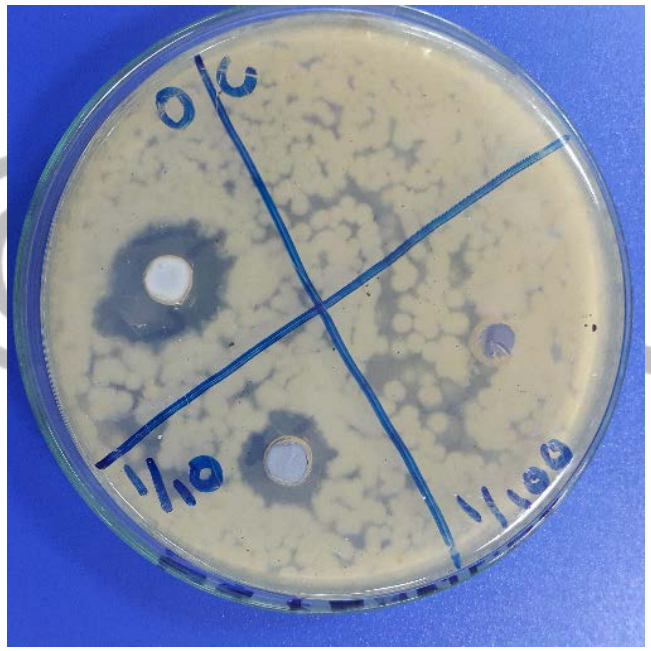

Fig 3.7: Anti-Fungal Activity on Day 7 at 2^{o}C-8^{o}C

Day 14

Table 3.5: Day 14-Room Temperature

	Anti-Bacterial			**Anti-Fungal**		
	Original	1/10th	1/100th	Original	1/10th	1/100th
Mean	13.7	9.3	0	10.0	0	0
	13.1	12.0	0	13.1	0	0
	13.2	9.7	0	12.0	0	0
	13.5	9.0	0	13.7	0	0
	13.375	10.0	0	12.2	0	0

Fig 3.8 shows that maximum anti-bacterial activity is exhibited in the original segment of the petri dish; it decreases to 10.0mm in 1\10th dilution and 0 in 1\100th dilution. Fig 3.9 shows that the zone of inhibition is only visible in the ditch with original preparation.

Fig 3.8: Anti-Bacterial Activity on Day 14 at 25^{o}C

Fig 3.9: Anti-Fungal Activity on Day 14 at 25^{o}C

Day 14-Fridge Temperature

	Anti-Bacterial			**Anti-Fungal**		
	Original	1/10th	1/100th	Original	1/10th	1/100th
Mean	12.65	0	0	19.0	11.4	0
	12.3	0	0	15.4	11.6	0
	11.55	0	0	14.6	10.6	0
	12.3	0	0	14.1	10.0	0
	12.2	0	0	15.775	10.9	0

Fig 3.10 shows a zone of inhibition in the partition with the original preparation and no inhibition in the other two areas. Fig 3.11 shows that the anti-fungal activity decreases with the decreasing concentration. It ultimately absent at day 14.

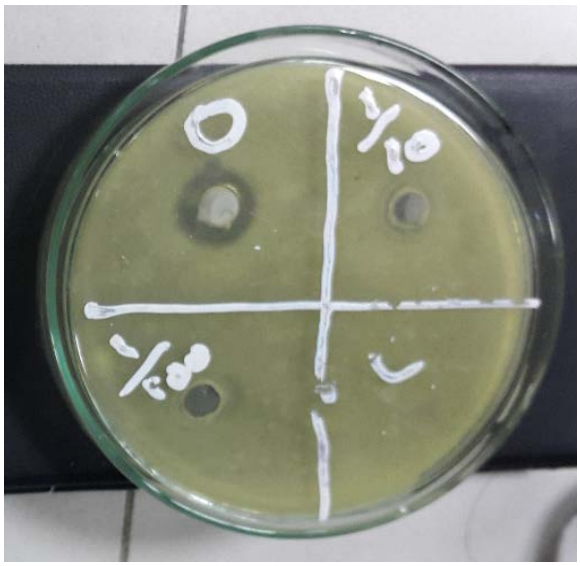

Fig 3.10 Anti-Bacterial Activity on Day 14 at 2^{o}C-8^{o}C

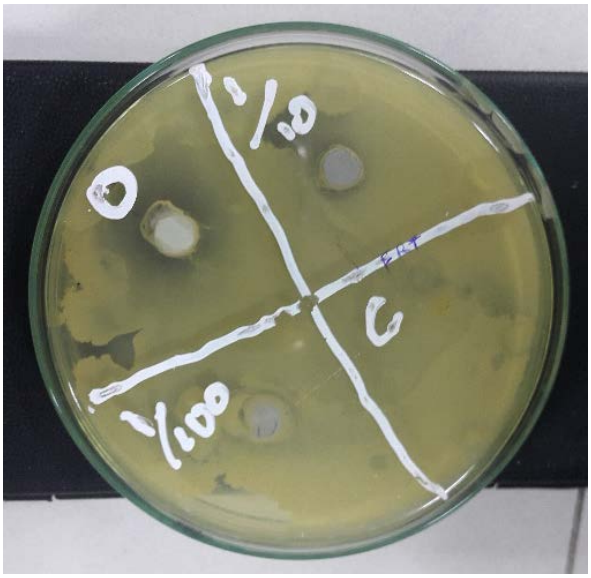

Fig 3.11: Anti-Fungal Activity on Day 14 at $2^{o}C$-$8^{o}C$

Discussion

Anti-bacterial Action

The anti-bacterial action of the preparation stored at room temperature, decreases with the decrease in concentration with the passage of time. When kept at the fridge temperature, the anti-bacterial activity decreases with decreasing concentrations. Although activity is lost for $1/10^{th}$ and $1/100^{th}$ dilution on day 14.

The antibacterial activity is higher than the antifungal activity at room temperature.

Anti-Fungal Action

The anti-fungal action occurs more with the decrease in concentration of a freshly prepared formulation, whereas as the time passes by the anti-fungal activity is only observed for the original formulation. This pattern is shown when stored at room temperature. The anti-fungal activity at fridge temperature, increases with the decrease in concentration on day 1. When observed from day 7 to day14, it shows a decreases in activity with decrease in concentration.

The antifungal activity is higher than the antibacterial activity in the original preparation.

Thus keeping in view the above results it can be said that the magic mouthwash shows maximum efficacy and stability in the original form and should be used within 15 days of preparation and also that it should be stored at room temperature for maximum usefulness. It is directed to shake well before use.

Conclusion

A stable mouthwash designed to treat oral mucositis caused by chemotherapy. There are various approaches to manage oral mucositis which involves the combination of different ingredients such as analgesics, local anesthetics and muco-protective agents. However, there is a likelihood of developing microbial colonization because of the disruption of mucosal membrane, therefore antifungals or antibacterial are added in the formulation

prophylactically, which may also treat other prevailing infection, if any. A well-known product, magic mouthwash was modified with the addition of chlorhexidine and checked for its physical stability, taste and efficacy in terms of antimicrobial activity at different storage conditions.

References

1. Bensinger, W., Schubert, M., Ang, K.K., Brizel, D., Brown, E., Eilers, J.G., Elting, L., Mittal, B.B., Schattner, M.A., Spielberger, R. and Treister, N.S., 2008. NCCN Task Force Report. prevention and management of mucositis in cancer care. *Journal of the National Comprehensive Cancer Network: JNCCN*, *6*, pp.S1-21.
2. Epstein, J.B. and Saunders, D.P., 2015. Managing Oral Mucositis Cancer Therapy.
3. Jones, J.A., Avritscher, E.B., Cooksley, C.D., Michelet, M., Bekele, B.N. and Elting, L.S., 2006.
4. Epidemiology of treatment-associated mucosal injury after treatment with newer regimens for lymphoma, breast, lung, or colorectal cancer. *Supportive care in cancer*, *14*(6), pp.505-515.
5. Lalla, R.V., Saunders, D.P. and Peterson, D.E., 2014. Chemotherapy or radiation-induced oral mucositis. *Dental clinics of North America*, *58*(2), pp.341-349.
6. Lalla, R.V., Bowen, J., Barasch, A., Elting, L., Epstein, J., Keefe, D.M., McGuire, D.B., Migliorati, C., Nicolatou-Galitis, O., Peterson, D.E. and Raber-Durlacher, J.E., 2014. MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. *Cancer*, *120*(10), pp.1453-1461.
7. McGuire, D.B., Fulton, J.S., Park, J., Brown, C.G., Correa, M.E.P., Eilers, J., Elad, S., Gibson, F., Oberle-Edwards, L.K., Bowen, J. and Lalla, R.V., 2013. Systematic review of basic oral care for the management of oral mucositis in cancer patients. *Supportive Care in Cancer*, *21*(11), pp.3165-3177.
8. Rosen, L.S., Abdi, E., Davis, I.D., Gutheil, J., Schnell, F.M., Zalcberg, J., Cesano, A., Gayko, U., Chen, M.G. and Clarke, S., 2006. Palifermin reduces the incidence of oral mucositis in patients with metastatic colorectal cancer treated with fluorouracil-based chemotherapy. *Journal of Clinical Oncology*, *24*(33), pp.5194-5200.
9. Saunders, D.P., Epstein, J.B., Elad, S., Allemano, J., Bossi, P., Van De Wetering, M.D., Rao, N.G., Potting, C., Cheng, K.K., Freidank, A. and Brennan, M.T., 2013. Systematic review of antimicrobials, mucosal coating agents, anesthetics, and analgesics for the management of oral mucositis in cancer patients. *Supportive Care in Cancer*, *21*(11), pp.3191-3207.
10. Vagliano, L., Feraut, C., Gobetto, G., Trunfio, A., Errico, A., Campani, V., Costazza, G., Mega, A., Matozzo, V., Berni, M. and Alberani, F., 2011. Incidence and severity of oral mucositis in patients undergoing haematopoietic SCT—results of a multicentre study. *Bone marrow transplantation*, *46*(5), pp.727-732.
11. Vera-Llonch, M., Oster, G., Hagiwara, M. and Sonis, S., 2006. Oral mucositis in patients undergoing radiation treatment for head and neck carcinoma. *Cancer*, *106*(2), pp.329-336.
12. Vera-Llonch, M., Oster, G., Ford, C.M., Lu, J. and Sonis, S., 2007. Oral mucositis and outcomes of allogeneic hematopoietic stem-cell transplantation in patients with hematologic malignancies. *Supportive Care in Cancer*, *15*(5), pp.491-496.