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Development and Evaluation of Medicated Mouth Wash

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Abstract

The present invention is to formulate and evaluate a novel medicated mouth rinse using Achyranthes aspera extract in an oil-based formulation. The herb Achyranthes aspera, which has long been used for medicinal purposes, was chosen because of its purported anti-inflammatory and antibacterial qualities. This study aimed to create a safe, effective, and pleasant-tasting mouth rinse to maintain oral hygiene and address common oral health issues. Achyranthes aspera's bioactive components were extracted and added to an oil-based carrier solution during the formulation process. To increase therapeutic potential while ensuring user acceptance, this novel mouthwash was enhanced with complementing natural essential oils. The evaluation study is based on Thin Layer Chromatography, pH, Gram staining, the determination of acid value and specific gravity. Additionally, research has found significant antimicrobial properties leading to their usefulness in treating common oral issues. This study advances the creation of a novel mouthwash that could be therapeutic by utilizing the oil-based medicinal properties of Achyranthes aspera. It is a plant species that is classified as a weed or wildflower. This plant can grow in tropical climates without needing to be started from seed. This plant is a weed, but it offers many, immediate, and unexpected health benefits. Eating Achyranthes seeds can aid in weight loss since they contain substances that help your body burn fat. This herb can also be very helpful for digestion. The formulation is bacteriostatic in nature which shows that it prohibits the growth of bacteria. It was made using Achyranthes aspera, which has capacity to reduce or prohibit dental caries. It is mainly based on an ancient method which is oil pulling. An alternative medicinal procedure called "oil pulling" involves swishing edible oil about the mouth for a while before spitting it out, much like mouthwash.

Keywords: *Achyranthes aspera*, anti-inflammatory, mouth rinse, natural, oral issues

INTRODUCTION

The plants have been used in traditional medicine for more than a millennium. In most underdeveloped nations, using traditional medicine is the normative foundation for maintaining good health. The finest source of treatment for a variety of illnesses and pains has historically been the plant kingdom. Because of this, medicinal plants have been essential to maintaining health on a global scale. The hunt for unique chemical variety and improvements in drug discovery techniques have accelerated efforts to explore potential leads from India's traditional medical system, Ayurveda [1]. The traditional applications of many plant products are taken into consideration in the modern era of drug research and the discovery of novel therapeutic compounds. *Achyranthes aspera* Linn. is one of the many plants whose medicinal efficacy is being investigated. It is a member of the Amaranthaceae family. It is an annual herb that is stiff and upright, and it is frequently seen growing as a weed throughout India [2]. According to the review, numerous phytochemical components have been isolated from the plant that have important medicinal properties, such as antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, and antiallergic. The root infusion can be used as a mild astringent for digestive issues, and the crushed

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plant is used to treat pneumonia. A decoction of powdered leaves with honey or sugar candy is beneficial when diarrhea and dysentery are still in the early stages. In recent years, a great deal of research has been done to show the biological activity and pharmacology of its extracts. Numerous chemical components have been identified, such as alkaloids, long-chain chemicals, dihydroxy ketones, saponins, oleanolic acid, and others [3].

Achyranthes aspera is a plant species that is classified as a weed or wildflower. This plant can grow in tropical climates without needing to be started from seed. This plant is a weed, but it offers many, immediate, and unexpected health benefits. Eating Achyranthes seeds can aid in weight loss since they contain substances that help your body burn fat. This herb can also be very helpful for digestion [4]. Due to this plant's incredible healing abilities for wounds, digestion, and body weight, many doctors still recommend it despite this negative effect. This plant has the potential to benefit a person's general wellbeing. Overall, this plant has been very helpful to many people's health, but you must seek a doctor's advice before utilizing this plant. *Achyranthes aspera* can treat a variety of health issues, including piles or fistulas, indigestion, renal calculus, cough, and cold, worms, cholera, wounds, and weight loss.

TAXONOMIC CLASSIFICATION [5]

- Kingdom – Plantae.
- Subkingdom – Tracheobinota.
- Super Division – Spermatophyta.
  - Division – Mangoliophyta.
  - Class – Mangoliophsida.
  - Subclass – Caryophyllidae.
- Order – Caryophyllales.
- Family – Amaranthaceae.
- Genus – *Achyranthes*.
- Species – *aspera*.

Taking care of your teeth and gums is crucial to your general health. In addition to causing gum disease and tooth cavities, poor oral hygiene can also cause diabetes, cancer, and heart problems. Maintaining healthy teeth and gums is a lifetime commitment. It will be simpler to prevent dental disorders and long-term health problems the earlier you start forming good oral practices. These dental practices include brushing, flossing, and consuming less alcohol and sugar-filled drinks [6].

Dental caries, another name for tooth decay, are damage to a tooth that can occur when oral bacteria that cause decay produce acid that attacks the tooth's enamel or surface. This may result in a cavity, which is a tiny hole or opening in a tooth caused by irreversible damage to the hard surface of your teeth. Cavities, also known as tooth decay or caries, are brought on by several things, such as oral bacteria, frequent snacking, sugar-filled beverages, and poor oral hygiene [7].

MATERIAL AND METHODS

Collection

a. Fresh leaves and roots of *Achyranthes aspera* were collected from Vadgaon bk, Pune district, India.
b. Saliva Sample was collected from Sinhgad Dental College, Vadgaon, and bacteria was grown and confirmed by Gram staining in laboratory.

Authentication of Plant

Plant authenticated from Agharkar Research Institute, Pune, Maharashtra, India.

Preparation of Extract [8]

Leaves, roots, and stems were separated.

- *For Leaves:* Leaves were dried at room temperature and grind in mixer, to obtain powder form. Extraction was carried out using hot Soxhlet Method. First, 15 g of leaf powder was defatted for 48

hours using petroleum ether. After the powder had been defatted, it was dried and extracted using ethyl acetate. To create an extract, the leftover powder was dried and extracted using chloroform. All the obtained three extracts were dried by the Rota Evaporator to carry out the dry extract.

- *For Stem:* Stem bark was macerated in petroleum ether and kept for 48 hrs.
- *For Root:* Root bark was macerated in petroleum ether and kept for 48 hrs.

PRELIMINARY PHYTOCHEMICAL INVESTIGATIONS

Phytochemical Studies [9]

Preparation for Mouth Rinse

Heat the base oil until it loses its froth or ceases to produce bubbles or froth. Take the oil off the burner. Now add the formulation's powder or extract in the designated ratio. To avoid burning or charring, constantly stir the basic oil during this procedure. Continue doing this until the base oil stops smoking. Once the medicinal oil is ready, filter it and rinse your mouth.

Formulation

So, we have prepared the mouth rinse (Figure 1). Firstly, for preparation of mouth rinse, we have taken three parts of *Achyranthes aspera*, i.e., leaf, root, and stem bark to ensure which part of plant shows more dental hygiene activities. So, we have taken three different concentrations of leaf powder, i.e., 2.5 g of leaf powder, 5 g of leaf powder and 10 g of leaf powder, all with sesame oil. We have taken three different concentrations of leaf powder because, if the leaf powder shows more dental medicinal property so to be precise how much concentration has more efficient function. Secondly, we have prepared the formulation using stem bark because; stem of *Achyranthes aspera* shows more medicinal property. And lastly, we used root bark to prepare the last formulation.

Figure 1. Medicated oil preparation.

STUDY OF ANTIMICROBIAL ACTIVITY [10]

Petri plates were sterilized in autoclave. Mitis Salivarius Agar was mixed in distilled water and poured on petri plates and were left to solidify. Bacterial culture (*Streptococcus mutans*) was inoculated in the agar medium in aseptic area. After this, all the petri plates were kept in an incubator for 48 hrs. (Temp: 35–40°C). Bacterial growth was observed and wells were made in agar to introduce the prepared mouth rinse as well as chlorhexidine as standard. 0.50 ml of mouth rinse was added into each well in aseptic conditions and the plates were again incubated for 48 hrs. Zone of inhibition was observed in most petri plates and was measured using Vernier caliper while comparing with zone of inhibition by standard solution (chlorhexidine).

Evaluation [11]

TLC: (Thin Layer Chromatography)

- The ideal plates were Silica Gel g. For TLC spots to form, a mobile phase consisting of a solvent combination was then created.
- A pencil was used to make a small mark on the bottom of the TLC plate. It facilitates the use of sample spots.
- Lastly, the chamber was filled with the stationary phase plate that had been prepared. After inserting the plate, the compartment was sealed.
- The plates were removed and given time to dry after the procedure.
- At last, the spots were analyzed through a UV light.
- After analyzing the compound Rf value was calculated.

pH: pH Was Calculated by Using Calibrated pH Meter [12]

Acid Value

- A suitable quantity of mouthwash was weighed into a 250 ml conical flask, to which 50 ml of ethyl alcohol and one or two drops of phenolphthalein indicator were also added.
- A color change was seen after the mixture was heated for five minutes and titrated against a potassium hydroxide solution.

Formula

V x 5.6 / Weight of sample

where V is the end point of titration.

Specific Gravity [13]

- An empty specific gravity bottle was weighed. Then it was filled with water and again weighed.
- Further different concentrations/extract mouth rinse were filled inside the specific gravity bottle and weighed.

Formula

$(W_3 – W_1) / (W_2 – W_1)$

where, W_1 – weight of empty bottle, W_2 – weight of bottle with water, W_3 – weight of bottle with mouth rinse.

RESULTS AND DISCUSSION

We performed TLC, by calculating distance traveled by each solute, i.e., formulation and distance traveled by solvent and using it we calculated Rf value. So, the results which we found that extract 1, i.e., PET ether has 0.288 Rf value. And the Rf value for extract 2, i.e., chloroform is 0.33. And the last extract 3, i.e., ethyl acetate has 0.4 Rf value.

Evaluation Parameters of Mouth Wash

pH of Formulation

For evaluating pH of the formulation, we have used pH meter and the results accordingly found are, F_1 (2.5 g leaf powder + Sesame oil) shows 4.5 pH value, F_2 (5 g leaf powder + Sesame oil) shows 4.3 pH value; F_3 (10 g leaf powder + Sesame oil) shows 4.4 pH value. The observation indicates that the mouth rinse is acidic in nature. Now coming towards F_4 (PET ether extract + Sesame oil) shows 4.3 pH value, F_5 (Chloroform extract + Sesame oil) shows 4.6 pH value and F_6 (Ethyl acetate extract + Sesame oil) shows 4.1 pH value. F_7 (Root bark + Sesame oil) shows 4.2 pH value, which shows it is acidic in nature and lastly F_8 (Stem bark + Sesame oil) shows 4.3 pH value, which shows that the formulation is acidic in nature.

Acid Value of Formulation

In medicated oils, maintaining low acid values is paramount to ensure that the oil remains safe and effective for use. The results which we found are, F_1 (2.5 g leaf powder + Sesame oil) shows 1.2%, F_2 (5 g leaf powder + Sesame oil) shows 0.8%; F_3 (10 g leaf powder + Sesame oil) shows 1.1%. Now coming towards F_4 (PET ether extract + Sesame oil) shows 0.9%, F_5 (Chloroform extract + Sesame oil) shows 1% and F_6 (Ethyl acetate extract + Sesame oil) shows 0.8%. F_7 (Root bark + Sesame oil) shows 0.9%, which shows it is acidic in nature and lastly F_8 (Stem bark + Sesame oil) shows 1.1%. These were the results which we got after testing it.

Specific Gravity of Formulation

The results which we found are, F_1 (2.5 g leaf powder + Sesame oil) shows 0.741, F_2 (5 g leaf powder + Sesame oil) shows 0.866; F_3 (10 g leaf powder + Sesame oil) shows 0.888. Now coming towards F_4 (PET ether extract + Sesame oil) shows 0.386, F_5 (Chloroform extract + Sesame oil) shows 0.505 and F_6 (Ethyl acetate extract + Sesame oil) shows 0.647. F_7 (Root bark + Sesame oil) shows 0.740, which shows it is acidic in nature and lastly F_8 (Stem bark + Sesame oil) shows 0.746. These were the results which we got after testing it.

TLC of Formulation

We performed TLC, by calculating distance traveled by each solute, i.e., formulation and distance traveled by solvent and using it we calculated Rf value. So, the results which we found are, for F_2A (2.5 g leaf powder + Sesame oil) shows 0.162, F_2B (5 g leaf powder + Sesame oil) shows 0.439; F_2C (10 g leaf powder + Sesame oil) shows 0.216. Now coming towards F_2D (PET ether extract + Sesame oil) shows 0.315, F_2E (Chloroform extract + Sesame oil) shows 0.307 and F_2F (Ethyl acetate extract + Sesame oil) shows 0.414. F_2G (Root bark + Sesame oil) shows 0.416, which shows it is acidic in nature and lastly F_2H (Stem bark + Sesame oil) shows 0.512 (Table 1, Figure 2(A–F)).

Table 1. TLC (thin layer chromatography).

Sample	Distance travelled by solute (A)	Distance travelled by solvent (B)	Rf- A/B
2.5 g leaf powder + Sesame Oil	A (1) – 0.6 A (2) – 1.3 A (3) – 2.5 A (4) – 3.0	B (1) – 3.7 B (2) – 3.7 B (3) – 3.7 B (4) – 3.7	Rf (1) – 0.162 Rf (2) – 0.351 Rf (3) – 0.675 Rf (4) – 0.810
5 g leaf powder + Sesame Oil	A (1) – 1.8 A (2) – 2.9 A (3) – 3.2	B (1) – 4.1 B (2) – 4.1 B (3) – 4.1	Rf (1) – 0.439 Rf (2) – 0.707 Rf (3) – 0.780
10 g leaf powder + Sesame Oil	A (1) – 0.8 A (2) – 1.7 A (3) –2.6	B (1) – 3.7 B (2) – 3.7 B (3) –3.7	Rf (1) – 0.216 Rf (2) – 0.459 Rf (3) – 0.702
Pet Ether + Sesame Oil	A (1) – 1.2 A (2) – 2.0 A (3) –2.9	B (1) – 3.8 B (2) – 3.8 B (3) –3.8	Rf (1) – 0.315 Rf (2) – 0.526 Rf (3) – 0.763
Chloroform + Sesame Oil	A (1) – 1.2 A (2) – 2.2 A (3) –3.4	B (1) –3.9 B (2) – 3.9 B (3) –3.9	Rf (1) – 0.307 Rf (2) – 0.564 Rf (3) – 0.871
Ethyl Acetate + Sesame Oil	A (1) – 1.7 A (2) – 3.0 A (3) –3.5	B (1) – 4.1 B (2) – 4.1 B (3) – 4.1	Rf (1) – 0.414 Rf (2) – 0.731 Rf (3) – 0.853
Root Bark + Sesame Oil	A (1) – 1.5 A (2) – 2.1 A (3) –2.8	B (1) – 3.6 B (2) – 3.6 B (3) –3.6	Rf (1) – 0.416 Rf (2) – 0.583 Rf (3) – 0.777
Stem Bark + Sesame Oil	A (1) – 2.1 A (2) – 2.6	B (1) – 4.1 B (2) – 4.1	Rf (1) – 0.512 Rf (2) – 0.634

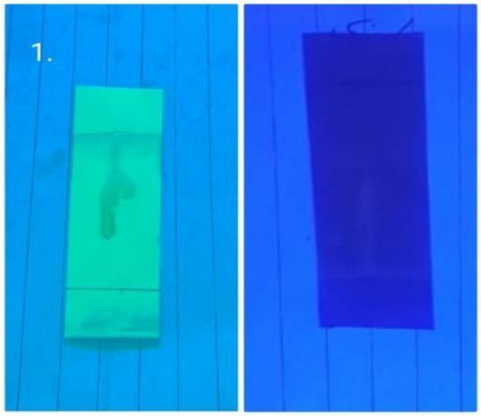


Figure 2(A). TLC study of 2.5 g leaf powder + sesame oil.

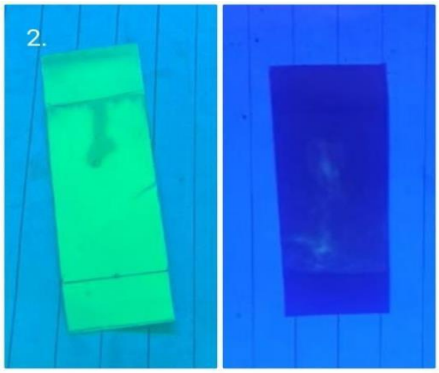


Figure 2(B). TLC study of 5 g leaf powder + sesame oil.

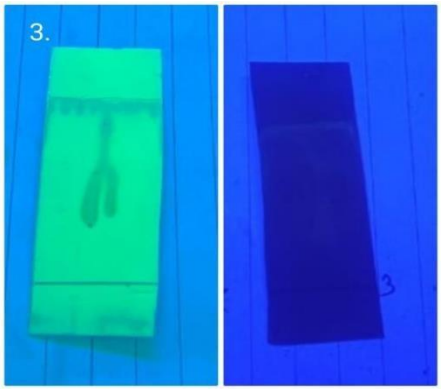


Figure 2(C). TLC study of 10 g leaf powder + sesame oil.

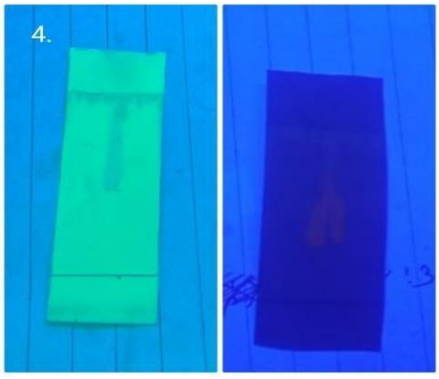


Figure 2(D). TLC study of PET ether + sesame oil.

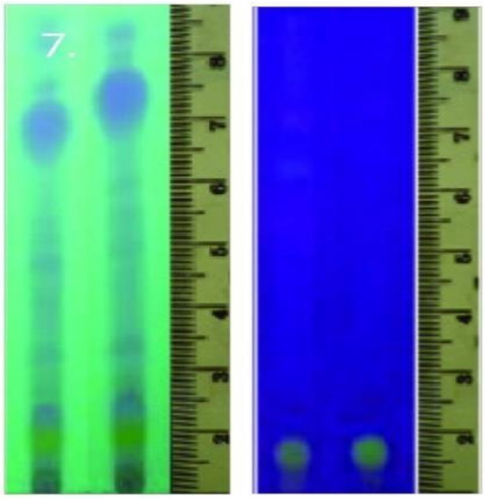


Figure 2(E). TLC study of root bark + sesame oil.

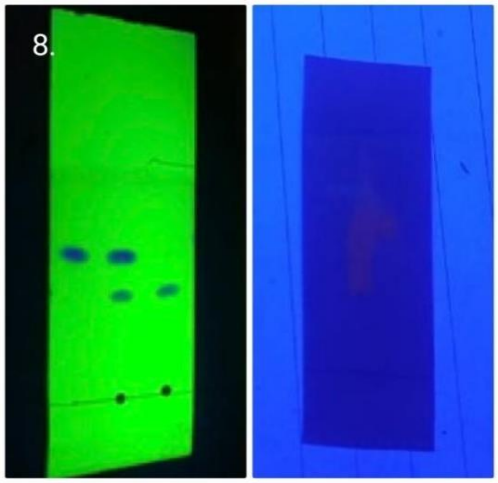


Figure 2(F). TLC study of stem bark + sesame oil.

Gram Staining Significance

We have taken four petri plates (Figure 3) and named it is petri plate (Figure 4). So, in petri plate 1, we had made four wells on it and named it is A1, B1, C1, and D1. In Figure 4(A–H), the growth of Staphylococcus mutans is shown on MBS agar. After the growth of bacteria, we had made four wells in each petri plate and named as A, B, C, and D. In petri plate 1, there are four wells A1, B1, C1, and D1. A1 well is empty, B1 well has been filled with chlorhexidine which is standard mouth rinse already present in market, in C1 well F_1 (2.5 g leaf powder + Sesame oil) was filled and D1 well had been filled with F_2 (5 g leaf powder + Sesame oil). As a result, the formulation made is bacteriostatic in nature as it prohibits the growth of bacteria. In petri plate 2, there are four wells A2, B2, C2, and D2. A2 well is empty, B2 well has been filled with chlorhexidine, C1 is filled with F_3 (10 g leaf powder + Sesame oil). D1 well had been filled with F_4 (PET ether extract + Sesame oil). As a result, also we found that the formulation made is bacteriostatic. In petri plate 3, there are four wells A3, B3, C3, and D3. A3 well is empty, B3 well has been filled with chlorhexidine, C3 is filled with F_5 (Chloroform extract + Sesame oil). D1 well had been filled with F_6 (ethyl acetate + Sesame oil). As a result, also we found that the formulation made is bacteriostatic. Lastly in petri plate 4, there are four wells A4, B4, C4, and D4. Like other petri plates, A4 well is empty, B4 well has been filled with chlorhexidine, C4 is filled with F_7 (root bark + Sesame oil). D4 well had been filled with F_8 (stem bark + Sesame oil). As a result, here also we found that the formulation made is bacteriostatic in nature.

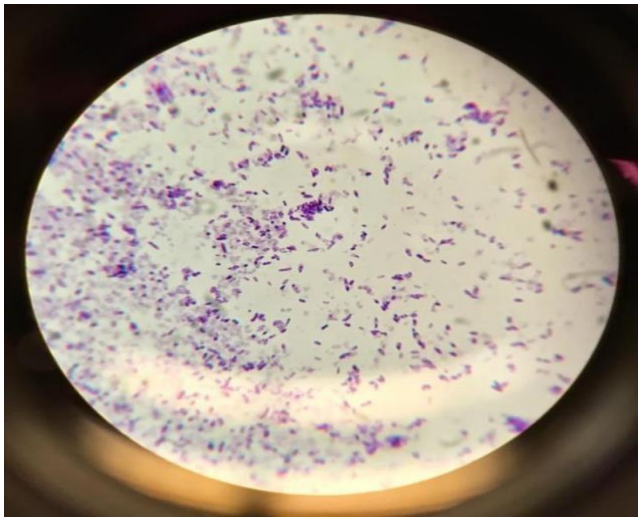
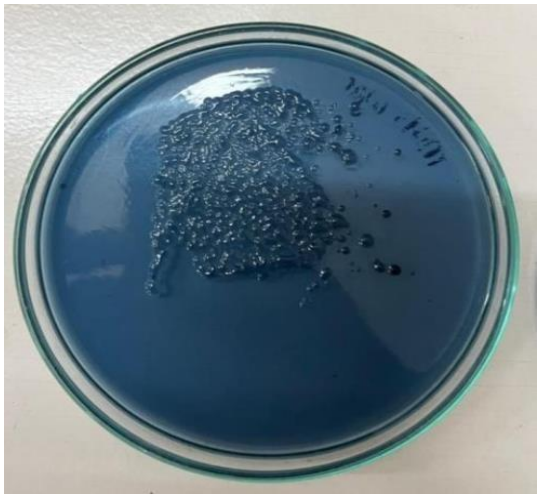
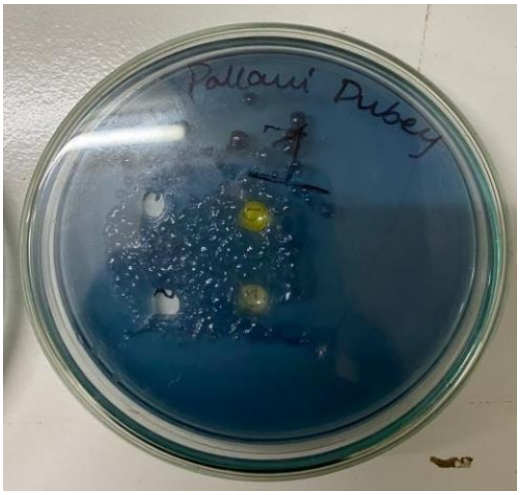


Figure 3. Microscopic view of staphylococcus mutans using gram staining.

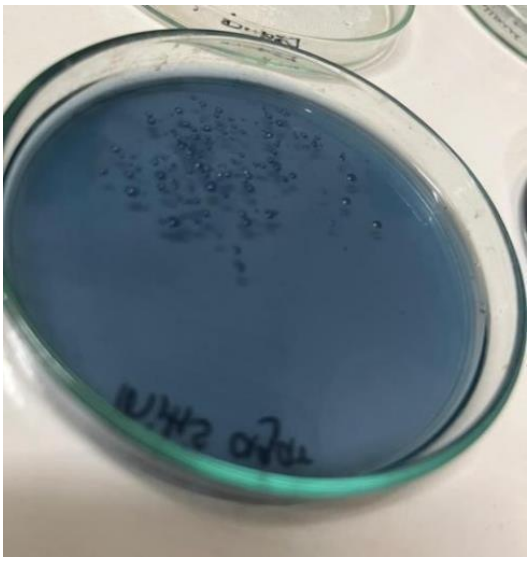
Growth of bacteria (*Staphylococcus mutans)* **Results**



(a). Petri plate 1 (growth)

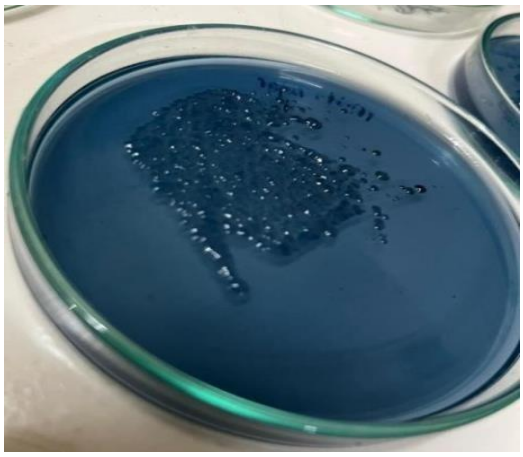


(b). Petri plate 1 (after introducingmouth rinse)

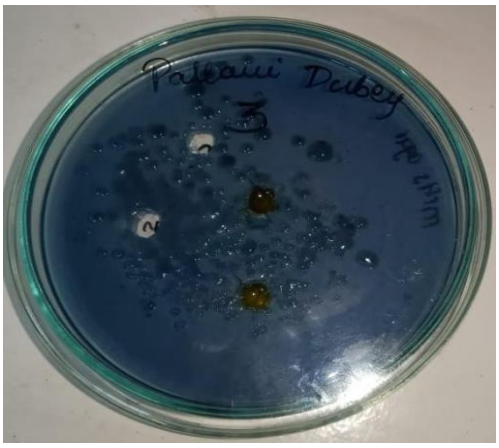


(c). Petri plate 2 (growth)

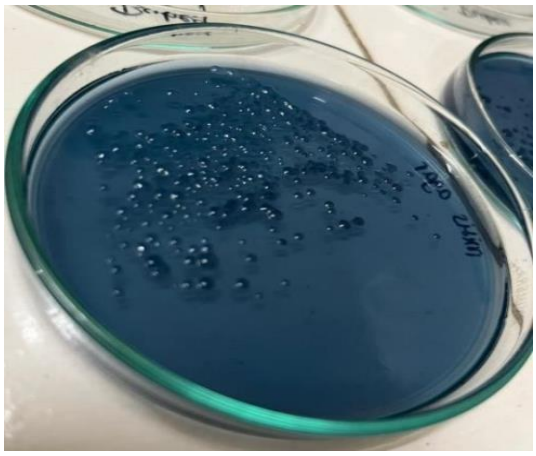
(d). Petri plate 2 (after introducing mouth rinse)



(e). Petri plate 3 (growth)



(f). Petri plate 3 (after introducing mouth rinse)



(g). Petri plate 4 (growth)

(h). Petri plate 4 (after introducing mouth rinse)

Figure 4. Antimicrobial activity.

SUMMARY AND CONCLUSION

By the end of the project, it was inferred from the result that mouth rinse as medicated oil was good in appearance, homogeneous, and easily applicable. It is found that formulation is bacteriostatic in nature which shows that it prohibits the growth of bacteria. It was made using *Achyranthes aspera*, which have capacity to reduce or prohibit dental caries. It is mainly based on an ancient method which is oil pulling. An alternative medicinal procedure called "oil pulling" involves swishing edible oil about the mouth for a while before spitting it out, much like mouthwash. The formulations prepared from *Achyranthes aspera* hence are bacteriostatic in nature.

Acknowledgments

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Conflict of Interest Statement

Author notified that experimental study is carried out without any financial or non-financial support for the procedures mentioned in the report.

REFERENCES

1. Dwivedi DS, Mehta KS. Achyranthes aspera Linn. (Chirchira): A magic herb in folk medicine. Ethnobot Leafl. 2008;12:670–676.
2. Vijai LB, Mahdi DS, Santosh KG. An overview of Achyranthes aspera Linn. J Sci Innov Res. 2018;7(1):27–29.

3. Saurabh S, Pradeep PP, Garima KK, Jha RL, Khosa RL. Achyranthes aspera—An important medicinal plant: A review. J Nat Prod Plant Resour. 2011;1(1):1–14.
4. Veena SV, Aastha GA, Urmila SC. Phytochemical investigation of various extracts of leaves and stems of Achyranthes aspera Linn. Int J Pharm Pharm Sci. 2013;5(1):27–32.
5. Azwanida NN. A review on the extraction methods used in medicinal plants: Principle, strength and limitation. Med Aromat Plants. 2015;4(3):11–16. doi: 10.4172/2167-0412.1000196.
6. Prashant GM, Chandu GN, Muralikrishna KS, Shafi Ulla MD. The effect of mango and neem extract on four organisms causing dental caries: Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis, and Streptococcus sanguis: An in vitro study. Indian J Dent Res. 2007;18(4):148–151. doi: 10.4103/0970-9290.35822.
7. Mandava K, Batchu UR, Kakulavaram S, Repally S, Chennuri I, Bedarakota S. Design and study of anticaries effect of different medicinal plants against S. mutans glucosyltransferase. BMC Complement Altern Med. 2019;19(1):197. doi: 10.1186/s12906-019-2608-3.
8. Palshikar GS. Environmental alterations on potency of eugenol content of basil: An antihypertensive herb. Acta Biol Slov. 2024;67(1):1–7. doi: 10.14720/abs.67.1.18390.
9. Sharma V, Agarwal A, Chaudhary U, Singh M. Phytochemical investigation of various extracts of leaves and stems of Achyranthes aspera Linn. Int J Pharm Pharm Sci. 2013;5(1):317–320.
10. Chimhete PN, Machona O, Mangoyi R. Preparation of an antibacterial herbal mouthwash containing extract of Erythrina abyssinica. Glob J Res Dent Sci. 2021;1(3):67–72.
11. Pinni J, Avula JS, Mukthineni S, Bandi S, Gokul T. Evaluation of anticariogenic efficacy of pomegranate (Punica granatum) pericarp extract as natural mouth rinse: An in vitro and in vivo study. Biomed Pharmacol J. 2018;11(4):2025–2030. doi: 10.13005/bpj/1578.
12. Srivastava PK. Achyranthes aspera: A potent immunostimulating plant for traditional medicine. Int J Pharm Sci Res. 2014;5(5):1601–1611. doi: 10.13040/IJPSR.0975-8232.
13. Ghimire K, Banerjee J, Gupta AK, Dahal P. Phytochemical constituents and pharmacological uses of medicinal plant Achyranthes aspera: A review. World J Pharm Res. 2015;4(1):470–489.

