

Neem And Peppermint Synergy: Advanced Mouth Wash Formulation and Evaluation for Oral Disease Prevention Like Gingivitis and Gum Infection

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Abstract—Oral diseases such as gingivitis, plaque formation, bad breath, and gum infections are among the most common health problems affecting people worldwide. The present study focuses on the formulation and evaluation of a polyherbal mouthwash containing neem and peppermint for effective oral disease prevention. Neem (*Azadirachta indica*) is well known for its antibacterial, antifungal, anti-inflammatory, and antiseptic properties, while peppermint (*Mentha piperita*) provides cooling sensation, refreshing flavour, and antimicrobial activity. The combination of these herbal ingredients was selected to develop a safe and effective alternative to synthetic mouthwashes, which may cause staining, irritation, and altered taste on prolonged use.

The mouthwash was prepared using suitable excipients and evaluated for various physicochemical parameters such as color, odor, pH, viscosity, stability, and homogeneity. Antimicrobial activity against oral pathogens was also assessed to determine its effectiveness in reducing microbial growth associated with gingivitis and gum infections. The formulated herbal mouthwash showed satisfactory stability, acceptable pH, pleasant taste, and significant antimicrobial activity. The study concludes that the synergistic effect of neem and peppermint can provide an effective, economical, and natural oral care formulation for maintaining oral hygiene and preventing periodontal diseases with

different varieties: Chemical and Natural compounds called polyphenols are found in mouthwashes and have the desired anti-inflammatory and anti-microbial properties. The demand for mouthwash is very high because they quickly relieve pain, fight oral pathogens, and have fewer side effects. They work without alcohol, artificial preservatives and coloring agents. Chemical mouthwashes typically contain hydrogen peroxide and chlorhexidine, which instantly whiten, sterilize, and relieve tooth pain. However, they have the tendency to discolor teeth and may cause side effects, meanwhile they are economical. Across the world, oral health is becoming a major concern. (1) The world oral health report, 2003, highlighted oral health as an integral and essential component of general health. Most of the chemical products contain an antiseptic that plays an important role in controlling plaque accumulation. The vehicles for delivery of chemical agents with antiplaque action are toothpaste, mouth washes, spray, irrigators, chewing gums and varnishes. However, mostly accepted method of delivering the antimicrobial agents after toothpaste is mouth wash. (2) Mouth wash is an antiseptic solution which is used to reduce the microbial load in the oral cavity. Many mouthwashes contain herbs with anti-microbial property such as neem, leaves, etc. some of the herbs that are used in mouthwashes are clove, which is traditionally used for oral health because of their antiseptic, antibacterial and antiviral property, peppermint which gives cooling effect to the mouth. Natural Herbs such as Tulsi, Green tea, Clove oil, Cinnamon oil and many others are used as single or in combination have been scientifically proven to be safe and effective medicine against oral health problems

I. INTRODUCTION

Mouthwash is an antiseptic aqueous solution used to clean the mouth and teeth or freshen up the breath. It is most often used for control of plaque and other dental problems. It is a medicated liquid that is held in the mouth and swished around by the perioral muscles to get rid of oral pathogens. Mouthwashes come in two

such as bleeding Gums, gingivitis and preventing tooth decay without side effects. Hence, most mouthwashes are safe alternative to pregnant women, peoples with dry mouth, diabetic and to children.

The purpose of this study was to determine the prevalence of mouthwash use and not only the type of mouthwash but quantity of mouthwash to be taken for use is also important and this study was performed to evaluate the efficacy and safety of mouthwash for human medicines. (3)

Label Of Mouthwash:

Active ingredient:	MOUTH WASH	Direction:
Neem	Promotes healthy gums and teeth.	After brushing
5ml		pour a capful of mouthwash
Mint		into your mouth swish thoroughly for 30
5ml		seconds then spit out.
Sucrose		
0.01g		
SLS		
.25g		
Sodium benzoate		Uses: kills germs that cause bad breath.
0.1g		
Glycerine	0	Helps to prevents plaque buildup.
3ml		Fight gingivitis. Fresh breath.
Ethanol		
5ml		
PEG		
1ml		
Water		
q.s		
Storage: Store in a cool, dry		Batch no: 0123
place. Keep away from		Mfg. Date:

		10/4/2026
sunlight.do not swallow.		Exp, Date: 10/5/2027
Caution: for external use		
only. Keep out of reach of		MRP. 40
Children		

Benefits of mouth wash (3)

- Mouthwashes can kill bacteria that causes bad breath.
- Mouthwashes can prevent plaque buildup and strengthen tooth enamel.
- Mouthwashes can kill bacteria that causes cavities, gum disease, and other infections.
- Mouthwashes can reduce gum inflammation.
- Some mouthwashes contain bleaching agents that can whiten teeth.
- Mouthwashes don't contain harsh chemicals like alcohol, triclosan, and sodium lauryl sulphate (SLS).
- Mouthwashes are gentle enough for people with sensitive teeth.

A. NEEM

- Synonym: Margosa tree, Indian lilac
- Biological Source: Neem is obtained from the tree *Azadirachta indica*
- Neem oil (from seed kernels)

Major active compounds include azadirachtin, nimbidin, and salannin.

- Family: Meliaceae

The World Health Organization refers to “Good-Health” as a state of physical and mental well- being not altered by any disease or ailment. Ancient Sanskrit had a particular expression for this state: “Nimba” which over time, derived into Neem. Nowadays, Neem is used to reference the *Azadirachta indica* (Neem) tree, traditionally though to bring “good health” to those who take them.(4) The Neem tree is primarily cultivated in the southern regions of Asia and Africa, where it has been seen used through many ages, in medical folklore. We should note that various parts of the Neem tree, including the leaves, bark, fruit, flowers, oil, and gum are associated with the medical folklore in the treatment of certain medical conditions such as cancer, hypertension, heart diseases, and diabetes. (5)



Fig.1: Azadirachta indica

The Neem tree is primarily cultivated in the southern regions of Asia and Africa, where it has been seen used through many ages, in medical folklore.(6) We should note that various parts of the Neem tree, including the leaves, bark, fruit, flowers, oil, and gum are associated with the aforementioned medical folklore in the treatment of certain medical conditions such as cancer, hypertension, heart diseases, and diabetes.(7) The potential effects that are seen when using these extracts can certainly be attributed cellular and molecular mechanisms, these mechanisms include free radical scavenging, detoxification, DNA repair, cell cycle alteration, programmed cell death mitigation and autophagy, immune surveillance, anti-inflammatory, anti-angiogenic, and anti-metastatic activities.(8)

Pharmacological Uses / Properties:

- Antibacterial
- Antifungal
- Antiviral
- Antimalarial
- Anti-inflammatory
- Insect repellent (widely used in organic farming and cosmetics)

B. MINT

- Synonym: Mentha, Peppermint
- Biological Source: Mentha piperita (Peppermint)

The aerial parts (mainly leaves and flowering tops) are used for medicinal, culinary, and cosmetic purposes. Main active constituents include menthol, menthone, and flavonoids.

- Family: Lamiaceae

Mint is one of the few medicinal plants, which are

widely cultivated for commercial purpose because of their immense pharmaceutical uses. (9) The farmers in tropical countries including India grow mints as an additional cash crop after rabi season optimizing the use of resources. Using fresh mint and other herbs and spices in cooking can help a person add flavour while reducing their sodium and sugar in take. (10) Throughout history, people have used different species of mint plants in medicine.



Fig. 2: Mentha piperita

All are herbaceous plants, readily sending out runners (rainy season) and stolons (winter), which develop new roots and shoots at the nodes and form plants. The entire aerial shoots together with foliage is a source of essential oil rich in menthol, carvone, linalool and linalyl acetate having use in pharmaceutical preparations and flavour industry. For the past four decades, mints are commercially cultivated in India. Of these, the Japanese Mint, yielding menthol is grown extensively in northern India. Other major producing countries are China and Brazil and to a smaller extent Thailand and Vietnam.

Pharmacological uses:

- Carminative (relieves gas and bloating)
- Antispasmodic
- Analgesic (especially topical for muscle pain)

Antimicrobial Flavoring agent (widely used in toothpaste, tea, and Food industry)

II. REVIEW OF LITERATURE

S.No.	Author	Year	Key Findings
1	Nigam D et	2026	Demonstrated

	al.		antimicrobial activity and effectiveness against oral infections
2	Shambharkar SB &Thakare VM	2026	Herbal mouthwash showed good efficacy with fewer side effects
3	Mariya Paul AN	2026	Highlighted safety and effectiveness of herbal oral care products
4	Rajesh Arora et al.	2026	Neem possesses strong antimicrobial and anti-inflammatory properties
5	Kalaskar AR et al.	2026	Effective in treating oral lichen planus and reducing symptoms
6	Kumar S & Kumar	2025	Showed enhanced protection against oral pathogens
7	Arshad MK et al.	2025	Mint has antimicrobial, antioxidant, and refreshing properties
8	Singh M et al.	2025	Strong antimicrobial effect and improved oral hygiene
9	Taheri JB et al.	2025	Herbal products beneficial in oral disease management

10	Taneja SC & Chandra S	2025	Mint improves taste and provides therapeutic benefits
11	Yuvashree CS et al.	2024	Showed antimicrobial activity with low cytotoxicity
12	Sakthivel A et al.	2024	Effective against oral pathogens and biofilm control
13	Vlachoianis C et al.	2024	Safe and effective for oral hygiene maintenance
14	Verma SK et al.	2024	Effective in reducing viral load in oral cavity
15	Janket SJ et al.	2024	Good oral hygiene linked with reduced cardiovascular risk
16	Khaky B et al.	2023	Prevents infections in ICU patients
17	Navabi N et al.	2023	Some chemical mouthwashes may cause side effects
18	Zhou F et al.	2022	Increasing research interest in herbal formulations
19	Natto ZS	2022	Herbs are effective in maintaining oral hygiene
20	Colledge WC	2022	Important for isolating

			active compounds in herbal formulations
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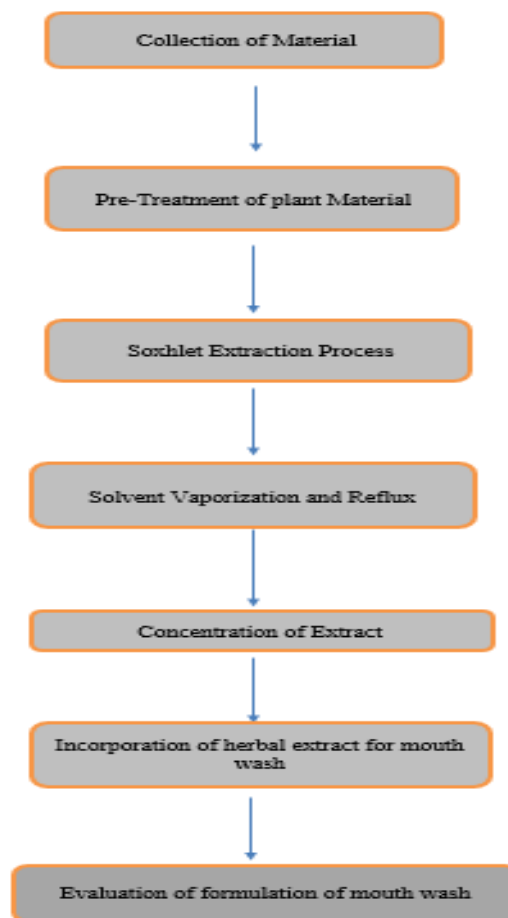
III. AIM AND OBJECTIVE

Aim: Neem and Peppermint Synergy: Advanced mouth wash formulation and evaluation for oral disease prevention like gum infection, gingivitis

Objectives:

1. To develop formulation of mouthwash: The present results therefore offer a greater use for traditional use of mouth wash.
2. Safety: Mouthwash was safe and there was neither report of adverse reactions.
3. Effectiveness: The objective of present work is to formulate and evaluate mouthwash and to evaluate its effectiveness against microbial load of oral cavity.
4. To maintain oral hygiene: Oral health is important as overall health. Now-a- days people may face more oral problems like periodontal disease, sore throat, gingivitis, plaque and so on. For maintaining good oral health various formulations are formulated.
5. Prevention, control and reduction of oral infection: It can reduce the plaque growth in your mouth, decrease your chances of developing gum disease, and prevent tooth decay.
6. To reduce side effects by promoting use: The use of herbs in dentistry should be based on evidence of effectiveness and safety. medicines, derived from botanical sources, have been applied in dentistry for a long history to inhibit microorganisms, reduce inflammation, soothe irritation, and relieve pain.

IV. PLAN OF WORK



V. METHODOLOGY: (11-15)

5.1. Collection of plant and preparation of extract: Fresh leaves of neem (*Azadirachta indica*) and peppermint (*Mentha piperita*) are collected and thoroughly washed to remove dirt and impurities. The cleaned leaves are shade-dried and then coarsely powdered. The powdered material is subjected to extraction using distilled water or a hydroalcoholic solvent by maceration or Soxhlet extraction. The extracts obtained are filtered and concentrated to form a semisolid mass.



Fig.3: Neem/Mint extract

5.2. Formulation of mouth wash:

For formulation, a measured quantity of neem and peppermint extracts is dissolved in purified water as the vehicle. Excipients such as glycerin (as a humectant), sodium benzoate (as a preservative), and saccharin sodium (as a sweetening agent) are added. The mixture is stirred continuously to ensure uniform distribution of all components. The pH of the formulation is adjusted to a suitable oral range (5.5–7). The final solution is filtered to remove any undissolved particles and stored in airtight containers.

Table no 1: Formulation of mouthwash: (16)

INGREDIENTS	FUNCTION	QUANTITY
Neem	Active drug	5ml
Mint	Active drug	5ml
Sucrose	Sweetener	0.01gm
SLS	Surfactant	0.25gm
Sodium benzoate	Preservative	0.1gm
Glycerin	Co surfactant	3ml
Ethanol	Solvent	5ml
PEG	Co surfactant	1ml
Distilled water	Solvent	Up to 50ml

5.3 Evaluation Parameters of Mouthwash:

1. Test for Alkaloids

The presence of alkaloids in the neem and mint extract was determined using standard qualitative tests. In Mayer's test, 2 mL of the extract was treated with a few drops of Mayer's reagent, which resulted in the formation of a cream-colored precipitate, indicating the presence of alkaloids.(16) In Wagner's test, the addition of Wagner's reagent to 2 mL of the extract produced a reddish-brown precipitate, confirming the presence of alkaloids.(17) Furthermore, Dragendorff's test was performed by adding Dragendorff's reagent to the extract, which yielded an orange or reddish-brown precipitate, thereby confirming the presence of alkaloids.(18)

2. Test for Flavonoids

Flavonoids in the extract were identified using different qualitative tests. In the alkaline reagent test, 2 mL of neem and mint extract was treated with sodium hydroxide solution, producing a yellow coloration that disappeared upon the addition of dilute hydrochloric acid, confirming the presence of flavonoids. (19–21) In the lead acetate test, the addition of a few drops of lead acetate solution to the extract resulted in a yellow precipitate, indicating

flavonoids. Additionally, in the ferric chloride test, the addition of FeCl_3 solution produced a blackish-green or blue coloration, which confirmed the presence of flavonoids. (22)

3. Test for Tannins

The presence of tannins was evaluated using several standard methods. In the ferric chloride test, the addition of FeCl_3 solution to 2 mL of the extract produced a blue-black or green coloration, confirming tannins. In the lead acetate test, a white or yellow precipitate was observed upon adding lead acetate solution, indicating tannins. The gelatine test also confirmed tannins by producing a white precipitate upon the addition of gelatine solution. (23) Moreover, the potassium dichromate test resulted in the formation of a brown precipitate, further confirming the presence of tannins.

4. Test for Phenols

Phenolic compounds were detected using standard qualitative tests. In the ferric chloride test, the addition of FeCl_3 solution to the extract produced blue, green, or violet coloration, indicating the presence of phenols. (24) In the lead acetate test, a white precipitate was formed, confirming phenolic compounds. Liebermann's test was also conducted by adding sodium nitrite followed by concentrated sulfuric acid, which resulted in a red or green coloration, thereby confirming the presence of phenols. (25)

5.4 Evaluation Parameters of Mouthwash:

1. Organoleptic Evaluation (Physical Appearance)

Organoleptic properties refer to the sensory characteristics of the mouthwash, which play an important role in patient compliance. These include color, odor, taste, and clarity. The mouthwash should have a pleasant color and should be free from turbidity or suspended particles, indicating good formulation stability. The odor must be refreshing, typically due to the presence of flavouring agents such as peppermint, while the taste should be acceptable and not too bitter or irritating. A clear and visually appealing mouthwash enhances user confidence and acceptability. (26)

2. pH Determination

The pH of a mouthwash is a critical parameter because

it directly affects the oral environment and tooth enamel. The pH is measured using a calibrated digital pH meter. Ideally, the pH of a mouthwash should be in the range of 5.5 to 7.5, which is compatible with the natural pH of saliva. A highly acidic mouthwash may lead to enamel erosion, whereas an alkaline pH may disturb the oral microflora. Therefore, maintaining an appropriate pH ensures both safety and effectiveness of the formulation. (27)

3. Viscosity

Viscosity determines the flow behaviour of the mouthwash and influences its ease of use. It is measured using a viscometer. A mouthwash should have low to moderate viscosity to allow easy swishing and proper distribution throughout the oral cavity. If the viscosity is too high, it may be difficult to use, whereas very low viscosity may reduce its retention time in the mouth, thereby decreasing its effectiveness. (28)

4. Antimicrobial Activity

One of the most important evaluation parameters of mouthwash is its ability to inhibit or kill oral microorganisms. The antimicrobial activity is tested against common oral pathogens such as *Streptococcus mutans*, *Lactobacillus*, and *Candida albicans*. Methods such as the agar well diffusion method are commonly used to measure the zone of inhibition. A larger zone indicates stronger antimicrobial activity. This parameter is essential in determining the effectiveness of the mouthwash in preventing oral diseases like plaque, gingivitis, and dental caries. (29)

5. Foamability Test

Foamability refers to the ability of the mouthwash to produce foam when shaken or used. It is evaluated by shaking a specific volume of the formulation in a graduated cylinder and measuring the height of the foam produced. Moderate foam formation is desirable as it enhances the cleaning action and user satisfaction. However, excessive foam may not be preferred in mouthwash formulations. (30)

6. Spreadability

Spreadability determines how well the mouthwash spreads within the oral cavity. Although it is more commonly evaluated in semi-solid formulations, it is

also relevant for liquid preparations to assess their coverage over oral surfaces. Good spreadability ensures that the active ingredients come into contact with all parts of the oral cavity, enhancing effectiveness. (31)

7. Taste Evaluation

Taste is a significant factor influencing patient compliance. The mouthwash should have a pleasant and refreshing taste, often achieved by adding sweeteners and flavouring agents such as menthol or peppermint oil. Bitterness or unpleasant taste may discourage regular use. Taste evaluation is usually conducted by a panel of volunteers under controlled conditions. (32)

8. Irritation Test

The irritation test is performed to ensure that the mouthwash does not cause any adverse effects such as burning sensation, redness, or irritation in the oral mucosa. This can be assessed through in vivo or in vitro methods. A safe formulation should be non-irritating and suitable for regular use. (33)

9. Anti Inflammatory Method:

a) Egg Albumin Denaturation Assay

Principle

One important aspect of inflammation is the denaturation of proteins. Anti-inflammatory activity is shown by the sample's capacity to prevent heat-induced albumin denaturation.

Materials Required

- Fresh egg albumin
- Phosphate buffer (pH 6.4)
- Test sample (patch extract)
- Standard: Diclofenac sodium
- Distilled water

- UV spectrophotometer

Procedure

0.2 mL of fresh egg albumin, 2.8 mL of phosphate buffer (pH 6.4), and 2 mL of the test sample at different concentrations made up the reaction mixture for the egg albumin denaturation test. The standard solution included diclofenac sodium, whereas a control was made using distilled water in place of the test sample. After 15 minutes of incubation at 37°C, each reaction mixture was heated to 70°C for five minutes in a water bath. A UV spectrophotometer was used to measure the absorbance at 660 nm after it had cooled to room temperature. The following formula was used to determine the percentage inhibition of protein denaturation. (34,36)

$$\% \text{inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Observation

* A higher percentage of inhibition denotes more potent anti-inflammatory action.

* Examine the exam against the standard.

VI. RESULT AND DISCUSSION

Table no. 2 A. Phytochemical Screening test of extract:

Active constituent	Neem extract	Mint extract	Observation
Alkaloids	Present	Present	Orange, brown, Precipitate
Flavonoids	Present	Present	Yellow, blackish green Precipitate
Tannins	Present	Present	Black, yellow, brown Precipitate
Phenols	Present	Present	Red, green Precipitate

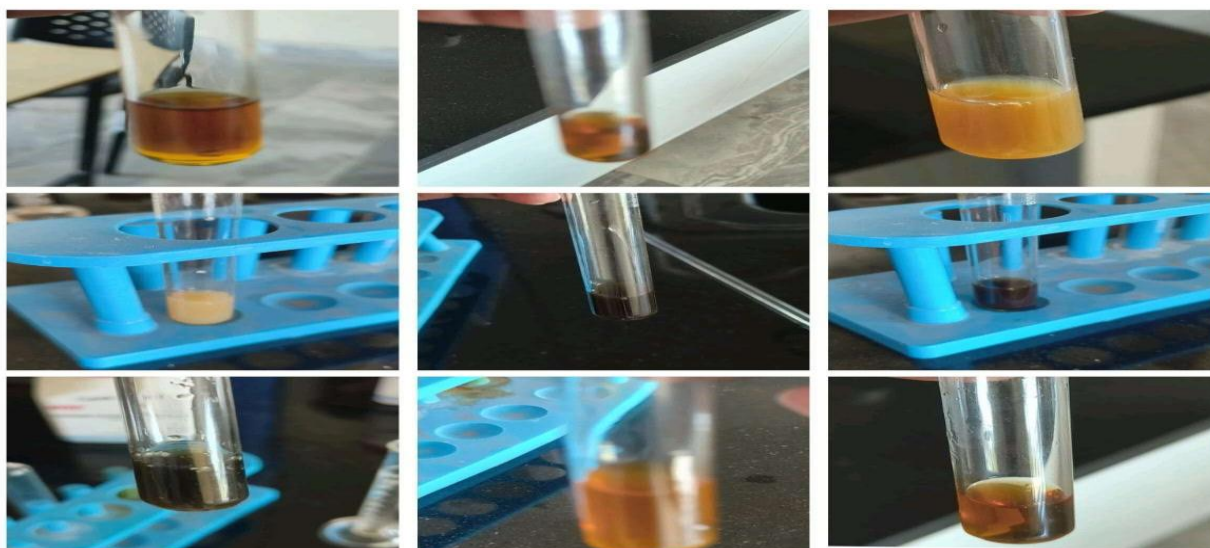


Fig. 4: Phytochemical tests of Neem/Mint

B. Evaluation parameters of mouth wash:**Organoleptic properties:**

Color: Yellow or greenish-yellow colour.

Odour: Slightly bitter.

Taste: Slightly bitter, slightly minty.

Discussion: The mouthwash was yellowish green in color with slightly bitter odour and taste due to neem and peppermint.

Ph: It showed a colour which detected the pH range 6.

Discussion: The pH of the formulated mouthwash was found to be around 6, which lies within the acceptable range for oral care product.

Viscosity measurement: It show us viscosity in the range of 35cp

Discussion: The viscosity of the formulated mouthwash was found to be _ cp using Ostwald viscometer at room temperature.

Irritation test: No redness, swelling, ulceration was observed in 24 hours.

Discussion: The mouthwash was found to be non-irritant and safe for regular oral use as no signs of irritation were observed.

Foamability: The height of foam produce was 1cm after 5 min.

Discussion: The mouth wash showed _ cm foam which disappeared with in 5 min, indicating good rinsing property and patient acceptability.

Anti-inflammatory activity:

The anti-inflammatory activity of the formulated neem and peppermint mouthwash was evaluated using the

protein denaturation method. In this test, 1 mL of mouthwash sample was mixed with 1 mL of bovine serum albumin solution. The pH of the reaction mixture was adjusted to 6.3 using 1N hydrochloric acid. The mixture was incubated at 37°C for 20 minutes and then heated at 57°C for 3 minutes. After cooling, the absorbance was measured at 660 nm using a UV-visible spectrophotometer. Diclofenac sodium was used as the standard anti-inflammatory drug, while distilled water served as the control. The percentage inhibition of protein denaturation was calculated using the formula:

Percentage Inhibition

$$= \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Percentage Inhibition

$$= \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Table no. 3 Discussion: As the concentration increases percentage inhibition also increases.

Concentration (µg/ml)	Percentage inhibition
100 µg/ml	45%
200 µg/ml	58%
300 µg/ml	67%
400 µg/ml	75%
500 µg/ml	83%

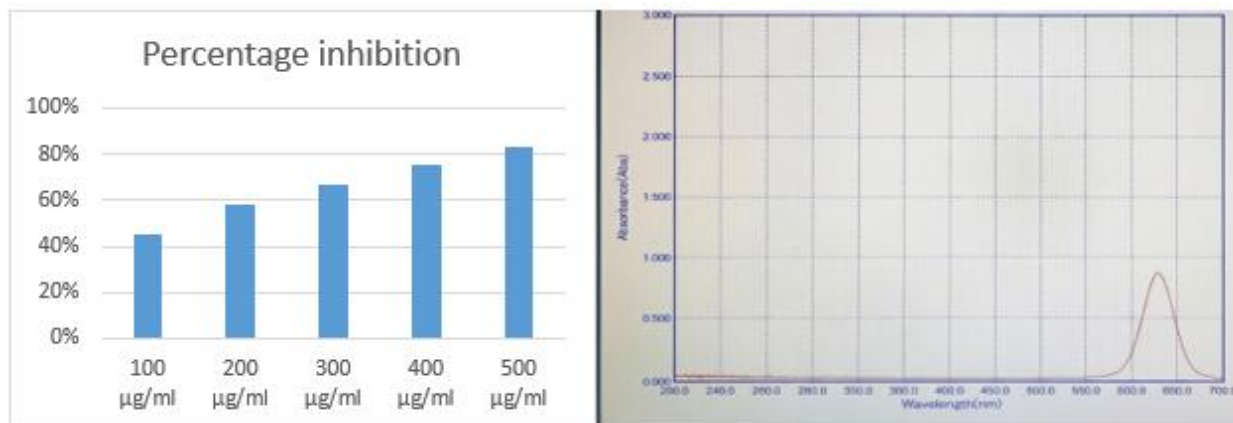


Fig. 5: UV of Mouth Wash Formulation

7. Anti-microbial activity:

The antimicrobial activity of the formulation of mouth wash containing neem and peppermint was evaluated using Agar well diffusion method.

Microorganism	Standard drug	Herbal formulation	Zone of inhibition of standard	Zone of inhibition of herbal formulation
Staphylococcus aureus	Chlorhexidine	Mouth wash (Neem+Peppermint)	22mm	17mm

VII. CONCLUSION

The present study successfully formulated and evaluated a mouthwash containing neem (*Azadirachta indica*) and mint (*Mentha spp.*) extracts for its potential effectiveness against oral infectious diseases. The mouthwash demonstrated significant antimicrobial activity, particularly against common oral pathogens such as *Staphylococcus aureus* and *Candida albicans*. Physicochemical parameters, including pH, viscosity and organoleptic properties, remained within acceptable limits throughout the evaluation period. These findings support the potential of neem and mint-based mouthwash as a natural, safe, and effective alternative to synthetic oral rinses. Further clinical studies are recommended to validate its long-term efficacy and safety in human subject.

The present liquid mouthwash can work in long way to help people to get rid of bad breath and many oral disorders. Besides we can be rest assured and take comfort in the fact that there aren't any unhealthy ingredients present in this preparation. The physicochemical evaluation results confirm that the colour and odour of present formulation is acceptable with a pleasant odour and a better aftereffect. The results of zone of inhibition also confirmed that this mouth rinses were found to be a potent plaque inhibitor, and were preferred by the patients for its taste, convenience of use and test duration in their mouth after rinsing. Thus, these can be used as an adjunct to mechanical therapy for treating plaque induced gingivitis. Present study has an important impact to create an effective and inexpensive oral health intervention for low social economic communities.

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