WWW.IJRPR.COM

**International Journal of Research Publication and Reviews**

Journal homepage: www.ijrpr.com ISSN 2582-7421

# Development, Formulation, And Evaluation Of The Antibacterial Properties Of A Herbal Toothpaste Prepared From The Seeds Of Chebulic Myrobalan

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**ABSTRACT:**

Medicinal plants are an integral part of human society in fighting against diseases. Chebulic myrobalan (Terminalia chebula), often referred to as the "King of Medicine" in Tibet and holds a prominent place in Ayurvedic medicine due to its excellent healing properties. The entire plant possesses significant medicinal value and has been traditionally used for the treatment of various diseases. The aim of the present study was to formulate antibacterial herbal toothpaste using Chebulic myrobalan. Fresh fruits were sourced from the local market in Haveri and authenticated by a taxonomist. The powdered fruit was extracted using 70% ethanol through maceration for 48 hours. Three toothpaste formulations (F1, F2, and F3) were prepared using varying concentrations of the extract. These formulations underwent evaluation for physical characteristics, pH, foamability, moisture content, and antimicrobial activity (zone of inhibition). Among them, the F3 formulation demonstrated superior physicochemical properties and exhibited the highest antibacterial activity, particularly against Escherichia coli.

**Keywords**: *Chebulic myrobalan*, Terminalia chebula, *Antibacterial herbal toothpaste*

## Introduction:

Herbal based toothpaste is widely used products since from ancient times. It plays a major role in the maintenance of oral health. The usage of toothpaste started in the year 500 BC at China and India. On that period, many of people uses crushed bones, powdered eggs and clam shells were used as abrasives, which are used for cleaning the teeth.(1) Multiple abrasives, fragrance agents, green lead were majorly utilized to remove strains from teeth until the middle of the 19th century

In modern-day, the addition of active ingredient during development and treatment of oral diseases has gained more important. Dentifrices, powder and paste are utilized to clean and improve the oral hygiene. In addition, the abrasive help in the removal and veiling of halitosis. The toothpaste releases the active ingredients like fluoride, which helps in the prevention of various teeth and gum diseases and primarily used to promote oral hygiene.

*Chebulic myrobalan* belongs to the family Combretaceae, The *Chebulic myrobalan* consists of 250 species and are widely distributed in tropical areas of the world. *Chebulic myrobalan* have various medicinal properties. The fresh leaves are used to cure bacterial infection, fungal infection and as vermifuge/pediculicide. Dried plant is used for fever, cough, asthma, urinary diseases, piles, worms and rheumatism. Roots are used for its astringent, purgative, stomachic, laxative and healing of wounds. Fruits are used for antibacterial and antifungal activities. Bark are used for heart disease, chest pain, high blood pressure, and high cholesterol and also used to increase sexual desire. *Chebulic myrobalan* contains different chemical constituents like tannins, phenolic acid, flavonoids, steroids, fatty acid etc.

As per WHO, herbal plants are widely used by 80% of the population as the primary healthcare treatment. Many synthetic formulations contain the chemicals and it causes tooth stain, altered taste and hypersensitivity reactions. So that, in search of alternative for the synthetic toothpaste, the herbal preparation plays a major role as they don't contain any artificial chemical agents such as sweeteners, odor and preservatives.(6,7) To produce effective herbal supplements with lesser side effects, the above factors are taken into consider.(8)

***Objectives:***

1. Formulate a antibacterial herbal toothpaste using *Chebulic myrobalan*
2. Conduct Pharmacognostic studies like collection and authentication of plant and Qualitative chemical identification tests of plant extract
3. Evaluation parameters of prepared herbal toothpaste like, Physical examination, $p^H$ , foamability, moisture content, antimicrobial activity ( zone of inhibition)

## Materials and Methods:

**List of Chemicals and Uses**

| Sl, No. | Materials | Uses |
|---|---|---|
| **1** | Calcium carbonate | Abrasive agent |
| **2** | Sodium lauryal sulphate | Detergent and foaming agent |
| **3** | Glycerine | Humecting agent |
| **4** | Tragacanth | Stabilizer |
| **5** | Saccharine | Sweetening agent |
| **6** | Peppermint oil | Flavoring agent |
| **7** | Citric acid | Preservative |
| **8** | Distilled water | Vehicle |
| **9** | Ethanol | Solvent |

***Collection of plant material:***

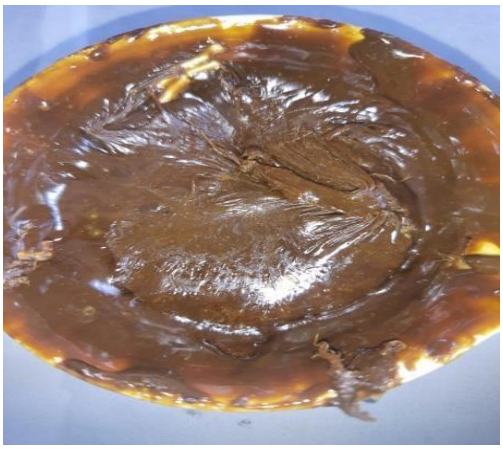
The fresh fruits of *Chebulic myrobalan* were collected from local market in Haveri and verified by taxonomist

***Chebulicmyrobalan Seeds and* Fruits**


***Preparation of Extract:***

*Chebulic myrobalan* fresh fruits are washed with sterile distilled water and air dried. Fruits were broken and seeds are collected and milled. 500 grams of milled *Chebulic myrobalan* powder is weighed into separate sterile beaker. The samples were extracted using 70% of ethanol and allow standing for 48 hours at room temperature with occasional shaking. The obtained suspensions were filtered into sterile beaker using muslin cloth. The filtrate obtained was then poured into china dish and air dried and the residue was collected.[17]

**Extract of *Chebulic myrobalan***



**Preformulation study:**

**Physicochemical properties of *Chebulic myrobalan*:**

- **Appearance**: Fine or coarse powder.
- **Colour**: Brown to dark brown or yellowish-brown.

- **Odour**: Mild, Characteristic herbal smell, sometimes slightly astringent.
- **Taste:** Bitter and astringent.
- **Category:** Antimicrobial, Antioxidant, Anti-inflammatory, Antiaging, and Rejuvenating properties.
- **Chemical constituents:** Tannins, phenolic acid, flavonoids, steroids, fatty acid etc.
- **Storage:** Store protected from light and moisture.
- **Solubility:** Moderately soluble in water, more soluble in alcohol-based solvents such as ethanol or methanol.

***Identification Tests:***

- **Test for Tannins:**

1. **Ferric chloride test:**
   To 2ml extract, add 5% ferric chloride solution drop by drop. Appearance of bluish black precipitate indicates the presence of tannins and phenolic compounds.
2. **Lead acetate test:**
   To 1ml extract, add 10ml of lead acetate solution. Appearance of white precipitate indicates the presence of tannins and phenolic compounds.
3. **Bromine water test:**
   Add aqueous bromine solution to the extract. Appearance of yellow precipitate indicates the presence of tannins

- **Test for flavonoids:**

1. **Shinoda test:**
   To the extract, a small piece of magnesium foil metal was added; this was followed by 3-5 drops of concentrated Hcl. The intense cherry red color indicates the presence of flavonoids.
2. **Alkaline reagent test:**
   2-3 drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HcL, indicating that flavonoids were present.

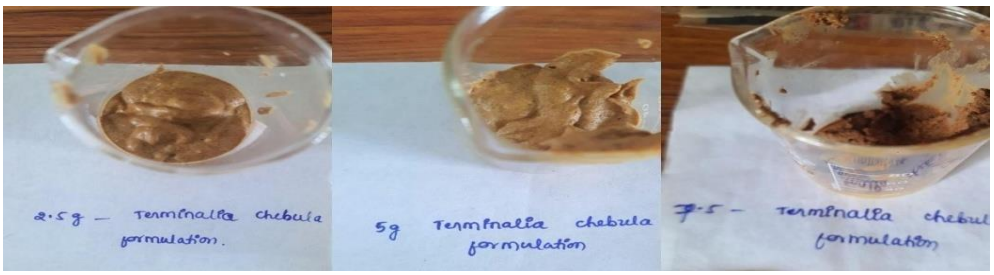
**FORMULATION TABLE**

| Sl No | Ingredients | F1 | F2 | F3 |
|---|---|---|---|---|
| **1.** | *Chebulicmyrobalan*extract | 2.5g | 5g | 7.5g |
| **2.** | Calcium carbonate | 10g | 10g | 10g |
| **3.** | Sodium lauryal sulphate | 0.75g | 0.75g | 0.75g |
| **4.** | Glycerine | 15mL | 15mL | 15mL |
| **5.** | Tragacanth | 0.6g | 0.6g | 0.6g |
| **6.** | Saccharine | 0.25g | 0.25g | 0.25g |
| **7.** | Peppermint oil | 0.5mL | 0.5mL | 0.5mL |
| **8.** | Citric acid | 0.5g | 0.5g | 0.5g |
| **9.** | Distilled water | 19.9mL | 17.4mL | 14.9mL |

***Preparation of Herbal Toothpaste:***

The ingredients required to prepare 50 g of toothpaste are listed in Table No. 3. To formulate the paste, tragacanth gum was first mixed with approximately 10 mL of distilled water in a mortar using a pestle. Glycerin was then added and triturated thoroughly. Calcium carbonate was incorporated gradually with continuous trituration to ensure a smooth consistency. The extract of *Chebulic myrobalan* was subsequently added and mixed thoroughly to achieve uniform distribution. Sodium lauryl sulfate (SLS) was incorporated with gentle stirring to minimize foam formation. Finally, saccharin, peppermint oil, and citric acid were added, and the mixture was adjusted to the final weight by adding distilled water

**Formulation of Herbal toothpaste**



**Evaluation of *Chebulic myrobalan* Toothpaste** [19]

**Physical examination**

Colour: Pale brown

Odour: Characteristic

Taste: Mint

**$p^H$ Determination:**

To make a 50% aqueous suspension, pour 10 grams of toothpaste from the container into a 50mL beaker and add 10mL of freshly boiled and cooled water (at 271$^0$C). Stir thoroughly and $P^H$ is determined by using $p^H$ meter for 5minutes.

**Foamability**:

Foamability was measured by mixing 2g of toothpaste with 5ml water in a measuring cylinder and then shake it for 10times. The total volume of foam was calculated by using the below formula.

Formability= Final volume–Initial volume

**Determination of Moisture Content:**

10g of toothpaste weighed in porcelain dish and dry in the oven at 105$^o$c. It was cooled in a room temperature. The loss of weight is recorded as percentage

moisture content and calculated by using the formula.

$$\% \text{ of moisture content} = \frac{Initial\ weight - final\ weigt}{Initial\ weight} \times 100$$

**Antibacterial activity (Zone of inhibition):**

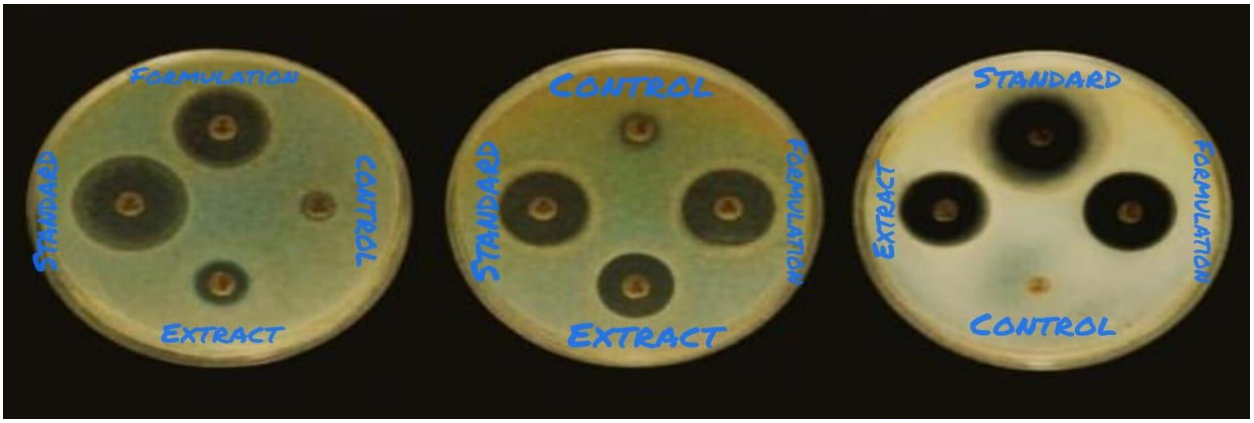
**Preparation of Microbial Culture:**

Take the 5g of peptone, 3g of beef extract, 5g of Sodium chloride, 15g of Agar were added to the 1000ml of distilled water in a flask. Later stir the components well until homogeneity and autoclave the solution at 121 $^0$C for 30minutes.To this solution 0.5ml of bacterial suspension was added and incubated for 24hours 40$^0$C.

**Preparation of Solid Agar Media:**

Take 20g of agar, 5g of beef extract, 10g of peptone transfer into the beaker which contains 100ml of water. Boil the solution until all the ingredients were dissolved. Sterilize the medium in hot air oven. Pour the prepared solution into the sterilized petriplates and allowed to solidify. After solidified make 4 zones in each petri plate using sterile crock borer and spread prepared bacterial culture using inoculation loop and keep it for one hour. Add the different concentration formulation, extract and a standard solution to the 3 zones and remaining one zone act as control. Keep the petri plates for incubation for 24hours at 37$^0$C. Test organism used for antibacterial activity is E.coli and observed the zone of inhibition and measures the diameter

**Antibacterial activity (Zone of inhibition)**



## RESULT AND DISCUSSION:

**Physical evaluation of formulation**

| Sl No. | Parameters | F1 | F2 | F3 |
|---|---|---|---|---|
| 1. | Colour | Light brown | Brown | Brown |
| 2. | Appearance | Paste | Paste | Paste |
| 3. | Texture | Smooth | Smooth | Smooth |
| 4. | Odour | Characteristic | Characteristic | Characteristic |
| 5. | Taste | Mint | Mint | Mint |
| 6. | Moisture content (%) | 18.22% | 19.15% | 20% |
| 7. | Foamability (cm) | 1.5cm | 2cm | 3cm |
| 8. | $p^H$ | **7.1** | **7.4** | 7.6 |

**Antimicrobial activity (Zone of inhibition)**

| Formulation | Formulation zone (mm) | Extract Zone (mm) | Standard Zone(mm) | Control Zone(mm) |
|---|---|---|---|---|
| **F1** | 15 | 12 | 20 | 10 |
| **F2** | 17 | 15 | 20 | 10 |
| **F3** | 19 | 18 | 20 | 10 |

## Discussion:

The phytochemical constituents present in the ethanolic extract of the *Chebulic myrobalan* contain tannins and flavonoids. The extract and standard shows high antibacterial activities against E. coli, whereas control not shown any antibacterial activity. The results of the sensory and physical evaluation of formulated herbal toothpaste containing different concentrations of *Chebulic myrobalan* extract (F1, F2 and F3) are presented in Table No4. F1 was light brown in colour, F2 and F3 was brown in colour. All formulations had a characteristic odour and a mint taste. All formulations were smooth in texture and paste in appearance. Moisture content of F1-18.22%, F2-19.15% and F3-20% Foaming ability of F1-1.5cm, F2-2cm and F3-3cm. $p^H$ of F1-7.1, F2-7.4 and F3-7.6. The F3 formulation shows high antibacterial activities against E.coli as compared to F1 and F2. Over all F3 formulation emerged as a most effective formulation in terms of physicochemical properties and high antibacterial activities against E. coli.

## Conclusion:

*Chebulic myrobolan* powder was extracted using 70% ethanol via maceration for 48hours and tested for phytochemical constituents and test results confirmed that it contains Tannins, phenolic acid, flavonoids, steroids, fatty acid etc. Antibacterial herbal toothpaste was prepared (F1, F2 and F3) by using different concentrations of extract of *Chebulic myrobolan*. The obtained results showed that F3 formulation emerged as a most effective formulation in terms of physicochemical properties and high antibacterial activities against E. coli. Toothpaste containing *Chebulic myrobolan* offers a multifaceted approach to oral hygiene, addressing not only microbial infections but also contributing to the overall maintenance of oral health, making it a viable and promising alternative to conventional oral care products

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