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DEVELOPMENT OF A MODIFIED DOSAGE FORM OF DASHANSANSKAR CHURNA AS A MOUTHWASH: PHARMACEUTICAL AND ANALYTICAL STANDARDIZATION WITH IN-VITRO ANTIMICROBIAL EVALUATION

***Dr. Amir Suhail[1], Dr. Vidushi Tyagi[2], Dr. Mohan C Joshi[2]**

[1]MD Final Year Scholar, Department of Rasa Shastra evum Bhaishajya Kalpana, Institute for Ayurved Studies and Research, Shri Krishna AYUSH University, Kurukshetra Haryana

[2]Guide and Head of Department, Department of Rasa Shastra evum Bhaishajya Kalpana, Institute for Ayurved Studies and Research, Shri Krishna AYUSH University, Kurukshetra Haryana

[3]MCARS, Jamia Millia Islamia, New Delhi, India-110025

***Corresponding Author's Email id -** amirsuhail184444@gmail.com

ABSTRACT

This study aimed to assess the antimicrobial efficacy and quality of a modified mouthwash prepared from the ingredients of Dashansanskar Churna by evaluating its raw materials and finished product through various analytical parameters. The determination of foreign matter confirmed the high purity of all ingredients, including Zingiber officinalis (Ginger) and Syzygium aromaticum (Clove). pH analysis revealed that most ingredients were slightly acidic, supporting their potential antimicrobial properties. Terminalia chebula (Haritaki) showed the most acidic pH (3.54). Total ash and acid-insoluble ash analyses demonstrated varying mineral content and minimal contamination, with Cyperus rotundus having the highest ash content (6.82%). Moisture content (loss on drying) was moderate, with Zingiber officinalis having the highest value (5.42%), influencing stability. The alcohol- and water-soluble extracts highlighted Terminalia chebula and Areca catechu as the main contributors

to the formulation's therapeutic properties. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) revealed diverse bioactive compounds across the raw drugs. The antimicrobial activity was tested against Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus, showing that Batches 5 and 6 had the highest efficacy. Batch 6 exhibited the broadest antimicrobial activity with inhibition zones up to 25 mm for S. aureus.

Keywords: Dashansanskar Churna, mouthwash, antimicrobial activity, pH analysis, TLC, HPTLC, bioactive compounds.

INTRODUCTION

Dashana Samskara Yoga is a well-documented formulation in Ayurvedic literature, renowned for its efficacy in addressing various oral cavity conditions.[1] Its therapeutic potential lies in its unique properties, including *Katu* (astringent) and *Tikta Rasa* (bitter taste); *Laghu* (light), *Rooksha* (rough), and *Tikshna* (strong) *Guna* (properties); *Katu Vipaka*; *Sheeta Veerya* (cold potency); and *Kapha Pitta Shamaka Karma* (action that balances *Kapha* and *Pitta*). These attributes, combined with its anti-inflammatory (*Shothahara*), styptic (*Lekhana* and *Shodhana*), and antimicrobial (*Krimighna*) properties, make it highly effective for managing gingival diseases.[2]

The classical preparation of *Dashana Samskara Churna*, as mentioned in *Bhaisajya Ratnavali Mukha Rogadhikara*, involves a combination of ten herbal and mineral ingredients, including *Sunthi*, *Haritaki*, *Musta*, *Kattha*, *Karpur*, *Supari Bhasma*, *Kalimarich*, *Lavang*, *Dalchini*, and *Khatika Churna* in specified proportions.[3] This formulation has been praised for its ability to alleviate oral ailments such as bleeding gums (*Sheetada*), pyorrhea (*Dantveshta*), tartar (*Dantasharkara*), and dental cavities (*Krimidanta*).[4]

The seven subsites of oral health described in *Ayurveda—Oshtha* (lips), *Dantamoola* (gingiva), *Danta* (teeth), *Jihva* (tongue), *Talu* (palate), *Kantha* (throat), and *Sarvasara* (oral mucosa)—are crucial for understanding oral diseases.[5] Notably, *Sheetada* (periodontal disease) is described in detail as a *Dantamoolagata Roga* in Ayurvedic texts. When untreated, this condition progresses from bleeding gums, discoloration, and sponginess to gum recession, tooth mobility, and suppuration, paralleling the modern understanding of gingivitis and periodontitis.[6]

The Ayurvedic practices of *Kaval* and *Gandusha* have long been employed to maintain oral health. *Kaval* involves swishing oil or medicated liquid in the oral cavity, while *Gandusha* requires holding a liquid without movement. These practices are therapeutic and preventive, strengthening oral structures and preventing decay, gum bleeding, and halitosis. Various types of *Gandusha*, such as *Snigdha* (unctuous), *Shamana* (pacifying), *Shodhana* (cleansing), and *Ropana* (healing), are tailored to specific oral and systemic conditions.[7]

Oral hygiene's role in systemic health is now well-established, with links between periodontal disease and systemic conditions like diabetes, cardiovascular disease, and adverse pregnancy outcomes. Maintaining oral health is essential, especially as bacterial infections in the oral cavity, caused by pathogens like *Streptococcus mutans*, are implicated in common dental issues such as caries, gingivitis, and periodontitis.[8]

Recent advances in Ayurvedic formulations focus on adapting traditional remedies into modern, user-friendly forms. This study aims to modify *Dashana Samskara Churna* into a mouthwash, leveraging its proven antimicrobial and anti-inflammatory properties. The study evaluates its pharmaceutical and analytical characteristics alongside its in-vitro antimicrobial efficacy, offering a promising natural alternative for oral healthcare.[9]

AIM & OBJECTIVES

AIMS:

To prepare and evaluate the mouthwash prepared from the ingredients of Dashan Sanskar churna.

OBJECTIVES:

1. Extraction of the constituents of Dashan Sanskar churna by Soxhlet extraction.
2. Preparation of the mouthwash from the extracts of Dashan Sanskar churna.
3. Assessing the in-vitro anti-microbial of the extract of Dashan Sanskar churna.

CHURNA KALPANA

Churna refers to a fine powder of a single drug or a mixture prepared through cleaning, drying, pounding, sieving (*vastragalita*), and uniformly mixing the ingredients.[10]

Historical Context: Referenced in Vedic texts such as *Atharvaveda* and *Yajurveda*. *Kalpana* signifies Ayurvedic preparation techniques.[11]

Preparation Method:

- Ingredients are cleaned, dried, powdered, sieved, and uniformly mixed.
- Aromatic drugs may be fried to enhance their aroma.

Mode of Administration:

- Mixed with *anupana* (e.g., honey, ghee, milk) for internal use.
- Applied externally as *Pratisaran* (rubbing on gums and teeth).

Preservation:

- Stored in airtight containers to avoid moisture and fungal growth.
- Shelf life: ~2 months, but potency may extend up to a year if stored properly.

Advantages:

- Easier dose fixation.
- Stable, economical, simple to prepare, and quick-acting when finely powdered.
- Convenient for transportation.

Limitations:

- Unsuitable for hygroscopic or volatile drugs.
- Unpleasant taste may reduce patient compliance, particularly in children.
- Shorter shelf life compared to other formulations.

Applications:

- Used for *Pratisaran* to enhance oral health.
- Forms the base for other formulations like *Vati* (tablets), *Avaleha* (herbal jams), and *Arka* (distillates).[12]

Concept of *Kaval* (Gargling)

Kaval involves holding or swirling oil or medicated liquids in the mouth to balance *doshas* (*Vata*, *Pitta*, *Kapha*), promote oral hygiene, and enhance systemic health.[12]

Significance

A key part of *Dinacharya* (daily regimen) in Ayurveda, *Kaval* highlights oral health as

essential for overall well-being. It prevents diseases and supports mental and physical balance.[13]

Classical References

- *Charaka Samhita*: Recommends gargling with honey, curd, ghee, and milk for oral and throat disorders.
- *Sushruta Samhita*: Highlights the use of medicated oils and decoctions to eliminate *doshas*.
- *Ashtanga Hridaya*: Notes the efficacy of herbal preparations for relieving *Vata*-related disorders.

Types of *Kaval*

1. **Snehana (Lubricating):** Moisturizes and nourishes; suitable for *Vata* imbalances.
2. **Shamana (Pacifying):** Soothes irritated tissues; useful for *Pitta* imbalances.
3. **Shodhana (Cleansing):** Detoxifies; addresses excess *Kapha*.
4. **Ropana (Healing):** Treats ulcers and promotes wound healing.

Procedure

- Prioritize oral hygiene before starting.
- Perform *Kaval* in the morning on an empty stomach.
- Swish the liquid gently in the mouth for 5–15 minutes until it thins and turns whitish, indicating toxin extraction.
- Spit out and rinse thoroughly with warm water.

Therapeutic Benefits

1. Strengthens teeth, gums, and facial muscles.
2. Reduces bad breath and prevents oral diseases.
3. Stimulates digestion and enhances voice clarity.

Precautions

- Avoid overuse to prevent dryness, ulcers, or fatigue.

- Follow prescribed guidelines for safe and effective practice.

Concept of *Gandusha*

Gandusha is an Ayurvedic therapeutic technique involving the retention of medicated liquids such as oils, herbal decoctions, ghee, or water in the mouth. The liquid is held without movement, filling the oral cavity entirely, and is selected based on the individual's *dosha* and specific health conditions.[14]

Significance

- Included in *Dinacharya* (daily regimen), *Gandusha* maintains oral health, prevents systemic diseases, and strengthens oral tissues.
- Practiced by healthy individuals using plain sesame oil (*Tila Taila*) or by diseased individuals with tailored medicated liquids, *Gandusha* cleanses the oral cavity and strengthens its defense mechanisms.[15]

Types of *Gandusha*

1. **Snigdha (Oleating):** Uses oily substances for dryness and *Vata* imbalance.
2. **Shamana (Palliative):** Cools and soothes *Pitta* imbalances like burning sensations.
3. **Shodhana (Purificatory):** Cleanses and detoxifies, addressing excess *Kapha*.
4. **Ropana (Healing):** Promotes healing of ulcers and wounds in the oral cavity.

Mechanism

- **Kashaya Gandusha:** Removes water-soluble toxins, preventing bacterial growth.
- **Sneha Gandusha:** Removes fat-based toxins and plaque, improving oral health.
- Stimulates salivary glands, balances oral pH, enhances circulation, and promotes healing.

Procedure

- Prepare by brushing teeth and tongue scraping.
- Sit upright in a calm environment, avoid wind or sunlight.
- Hold the liquid until secretions appear from the throat, nose, or eyes, then spit it out.
- Repeat 3–7 times as needed.

Benefits

1. Strengthens teeth, gums, and jaws.
2. Prevents dryness, cracking lips, and dental caries.
3. Enhances taste perception and immunity.
4. Promotes healing of oral ulcers and wounds.

Assessment of *Gandusha*

- **Proper Effects (Shuddha):** Alleviation of illness, oral freshness, lightness, and improved sensory clarity.
- **Inadequate Effects (Heena Yoga):** Stiffness, excessive salivation, and reduced taste.
- **Excessive Effects (Ati Yoga):** Mouth ulcers, dryness, thirst, and exhaustion.

Indications

- Treats oral conditions like bleeding gums (*Sheetada*), dental cavities (*Krimidanta*), gum diseases (*Dantaveshta*), tartar (*Dantasharkara*), and stomatitis (*Mukhapaka*).
- Useful in head and neck diseases like rhinitis (*Pinasa*), throat abscess (*Gala Vidradhi*), and diphtheria (*Kantharohini*).

Contraindications

- Avoid in children under five, bleeding disorders, emaciation, and acute conditions like rhinitis or indigestion.

Drug Review

Dashan Samskar Churna

An Ayurvedic formulation for oral health documented in *Bhaisajya Ratnavali* and AFI Part-II. It comprises *Sunthi*, *Haritaki*, *Musta*, *Khadira*, *Karpur*, *Supari Bhasma*, *Kalimarich*, *Lavanga*, *Dalchini*, and *Khatika Churna*.[16]

Therapeutic Applications:

- Treats conditions like *Sheetada* (bleeding gums), *Dant Veshta* (pyorrhea), *Dantasharkara* (tartar), and *Krimidanta* (dental cavities).

Pharmacological Properties:

- Balances *Vata*, *Pitta*, and *Kapha doshas*.
- Possesses anti-inflammatory, antimicrobial, and detoxifying effects.

Shelf Life: Approximately 2 months.

Key Benefits:

- Strengthens teeth and gums.
- Reduces inflammation and microbial load.
- Promotes overall oral hygiene.

Table No. 2: Showing the properties of ingredients of Dashansanskar Churna

Ingredients	Rasa	Guna	Virya	Vipaka	Karma	Part used	Quantity
Khadir[17] (Acacia catechu)	Tikta, Kashaya	Laghu ruksha	Sheeta	Katu	Kapha-pittashamak Krimighnadantya	Heart-wood Bark	1 Part
Karpura[18] (Cinnamomum camphora)	Katu tikta Madhur	Laghu tikshna	Sheeta	Katu	Tridhoshara Mukhdurgandhnasak	Extract	1 Part
Kramuk[19] (Areca catechu)	Kashaya Madhur	Ruksha guru	Sheeta	Katu	Kapha-pittashamak mukhvairasyanashan	Fruit seed	1 Part
Shunti[20] (Zingiber officinalis)	Katu	Snigdha Laghu	Ushna	Madhu	Vata-kaphashamak Aamdoshahar	Rhizome	1 Part
Haritaki[21] (Terminalia chebula)	Madhur, amla, katu, tikta, Kashaya	Laghuruksha	Ushna	Madhur	Sarvadoshpra shamanrasayan, chakshushya	Fruit pulp	1 Part
Musta[22] (Cyprus rotundus)	Tikta katu Kashaya	Laghu ruksha sheeta	Sheeta	Katu	Kapha-pitta shamak	Rhizome	1 Part
Maricha[23] (Piper nigrum)	Katu tikta	Laghu ruksha	Ushna	Katu madhur	Kapha-vatashamaka	Fruit	1 Part

		tikshna guru					
Lavanga[24] (Syzygium aromaticum)	Katu tikta	Vishad ruksha laghu tikshna	Sheeta	Madhur	Kapha-vatashamaka	Flower bud	1 Part
Twak[25] (Cinnamomum zeylanicum)	Madhur katu tikta	Ruksha laghu tikshna ushna	Sheeta ushna	Katu	Vata-kaphashamaka	Bark	1 Part
Khatika[26] churna ($Caco_3$)	Tikta Madhur	Sheeta			Shothahara, Pittashamaka	Churna	9 Part

MATERIAL AND METHODS

Material

The materials used for the preparation of Dashansanskar mouthwash were meticulously chosen and sourced from reliable suppliers to ensure the quality and efficacy of the final product. The list of materials includes raw herbal ingredients, solvents, and equipment.

1. Raw Herbal Ingredients

The following herbs and materials were procured for the formulation of Dashansanskar mouthwash:

Sr. No.	Ingredient	Latin Name
1	*Shunthi*	*Zingiber officinalis*
2	*Khadir*	*Acacia catechu*
3	*Supari*	*Areca catechu*
4	*Haritaki*	*Terminalia chebula*
5	*Lavang*	*Syzygium aromaticum*
6	*Twak*	*Cinnamomum zeylanicum*
7	*Maricha*	*Piper nigrum*
8	*Musta*	*Cyperus rotundus*
9	*Karpur*	*Cinnamomum camphora*
10	*Khatika Churna*	Calcium carbonate

Solvents Used:

- High-Performance Liquid Chromatography (HPLC) Grade Water
- Ethanol (used in a 70:30 ratio with water for hydro-alcoholic extracts)

2. Equipment

The preparation process required a range of specialized laboratory equipment to ensure proper extraction and formulation:

- **Soxhlet Extractor**: For continuous and efficient extraction of bioactive compounds.
- **Heating Mantle**: Maintains controlled temperatures between 55–110°C during extraction.
- **Centrifugal Machine**: Operated at 12,000 rpm for 15 minutes to separate impurities.
- **Measuring Cylinders**: For precise measurement of solvents and liquid extracts.
- **Whatman Filter Paper (Nos. 41 and 44)**: Ensures fine filtration of the extracts.
- **Beakers and Plastic Tubes**: For mixing and transferring liquids during the formulation process.
- **Stirrer**: For homogenizing the mouthwash formulation.
- **Weighing Machine**: For accurate measurement of raw materials and ingredients.

3. Procurement Details

- **Suppliers**:
    - Shri Ram Herbal, Jaipur, Rajasthan.
    - Panna Lal Brij Lal Herb Shop, Haridwar, Uttarakhand.
- **Authentication**:
    - Conducted by CARI (CCRAS, Jhansi) and PG Department of Dravya Guna, Institute for Ayurvedic Studies and Research, Kurukshetra.
    - Verified based on morphological characters mentioned in Ayurvedic Pharmacopoeia of India (API).

Methods

The preparation of mouthwash from the ingredients of Dashansanskar churna (6 Batches) involved standardized procedures for the extraction of active components and formulation of the final product. The following methods were employed:

1. Preparation of Extracts

Aqueous Extract

1. **Selection and Preparation of Raw Material**:
    - The herbs were cleaned to remove impurities and dried under shade.
    - Dried herbs were powdered using a sieve no. 10 for uniform particle size.
2. **Extraction Process**:
    - **Quantity Used**: 20 g of each herb was taken.
    - **Solvent**: 200 ml of distilled water for each herb.
    - **Procedure**:
        - The powdered herbs were placed in the Soxhlet extractor.
        - Water was heated in a round-bottom flask using a heating mantle, maintaining a temperature of 70–80°C.
        - The solvent vaporized, condensed, and continuously passed through the herb material in cycles (8–10 cycles).
        - The process was run for 4–5 hours.
3. **Filtration and Concentration**:
    - The extract was filtered using Whatman filter paper no. 41.
    - The filtrate was concentrated by evaporating excess water using a rotary evaporator.
4. **Final Product**:
    - A concentrated aqueous extract was obtained and stored in airtight containers.

Hydro-Alcoholic Extract

1. **Preparation of Solvent**:
    - A mixture of ethanol (30%) and water (70%) was prepared.
2. **Extraction Process**:

- Similar to the aqueous extraction process, 20 g of each herb was used with 140 ml of water and 60 ml of ethanol.
- The Soxhlet extraction method was employed, maintaining a temperature of 70–80°C.
- The process ran for 8–10 cycles over 4–5 hours.

3. **Filtration and Concentration**:
    - The hydro-alcoholic extract was filtered and concentrated to remove the solvent mixture.
4. **Final Product**:
    - The concentrated hydro-alcoholic extract was stored in airtight containers.

2. Formulation of Mouthwash prepared from the Dashansanskar Churna

1. **Preparation of Ingredients**:
    - 5 g of each herb powder was mixed for the aqueous and hydro-alcoholic formulations.
    - 2 g of *Karpur* (camphor) and 5 g of *Khatika churna* (calcium carbonate) were separately dissolved in water and filtered using Whatman filter paper no. 44.
2. **Mixing and Homogenization**:
    - The prepared extracts (aqueous or hydro-alcoholic) were combined with the filtered *Khatika churna* solution.
    - The mixture was homogenized using a mechanical stirrer to ensure uniform consistency.
3. **Filtration**:
    - The final mixture was filtered again to remove any undissolved particles.
4. **Storage**:
    - The prepared mouthwash was stored in sterile, airtight containers to prevent contamination.

3. Quality Control Measures

1. **pH Testing**:
    - The pH of the mouthwash was checked to ensure it was within the acceptable range for oral use.
2. **Stability Testing**:
    - The stability of the product was tested under different conditions (temperature and humidity).
3. **Microbial Testing**:
    - The product was evaluated for microbial contamination in a certified laboratory.
4. **Organoleptic Properties**:
    - The mouthwash was tested for its taste, color, and odor to ensure consumer acceptability.

4. Precautions

- Equipment was sterilized before and after use.
- All processes were conducted under aseptic conditions.
- The product was stored in airtight containers to maintain its stability and efficacy.

Analytical Study and Results

Summary Table of Analytical Study

Parameter	Observation	Significance
Foreign Matter	All raw materials (e.g., *Zingiber officinalis*, *Piper nigrum*, *Syzygium aromaticum*) were free from contaminants (0.00%).	Ensures high quality and purity of the ingredients, crucial for therapeutic efficacy and safety.
pH Analysis	Ingredients were slightly acidic; *Terminalia chebula* had the lowest pH (3.54).	Supports antimicrobial properties and oral hygiene.
Total Ash Content	Varied among ingredients; highest in *Cyperus rotundus* (6.82%) and lowest in *Acacia catechu* (1.41%).	Reflects mineral composition, contributing to the formulation's properties.

Loss on Drying	Moderate moisture content; highest in *Zingiber officinalis* (5.42%).	Affects stability and shelf life of the formulation.
Acid-Insoluble Ash	Minimal contamination; *Acacia catechu* had the lowest content (0.01%).	Demonstrates absence of non-organic impurities, ensuring quality.
Alcohol-Soluble Extract	High in *Terminalia chebula* (44.73%) and *Areca catechu* (42.50%).	Contains bioactive compounds crucial for antimicrobial and healing properties.
Water-Soluble Extract	High in *Terminalia chebula* (62.29%) and *Areca catechu* (38.25%).	Includes antioxidant and cleansing agents, enhancing oral hygiene.
TLC Results	Unique Rf values across raw drugs confirmed diverse bioactive compounds (e.g., eugenol in *Syzygium aromaticum* and tannins in *Terminalia chebula*).	Demonstrates chemical diversity and therapeutic potential of the formulation.
HPTLC Results	Separation of bioactive compounds confirmed at 254 nm and 366 nm wavelengths.	Validates the presence of active ingredients and supports quality control.
Antimicrobial Activity	- **Batch 6:** Strongest activity (e.g., 25 mm inhibition zone against *S. aureus*). - **Batch 5:** Significant activity (e.g., 17 mm inhibition zone).	Confirms effectiveness of the mouthwash formulation, especially Batch 6, against various microorganisms.

Phenols, Flavonoids Standard, Alkaloids Standard

Table no. 1: Phenols

Sr. No.	Concentration (conc.)	Absorbance at 470 nm
1	5	0.653
2	15	0.753
3	25	1.011
4	35	1.387

Table no. 2: Flavonoids

Sr. No.	Concentration (conc.)	Absorbance
1	10	0.106
2	20	0.147
3	30	0.173
4	40	0.199

Table no. 3: Analytical Study and Results

Sr. No.	Concentration (conc.)	Absorbance
5	50	0.242
6	60	0.277

Table no. 4: Alkaloids Standard

Sr. No.	Concentration (conc.)	Absorbance
1	20	0.083
2	40	0.112
3	80	0.156
4	100	0.201
5	120	0.249

DISCUSSION

Foreign Matter

The analysis confirmed the absence of foreign matter (0.00%) in all raw materials, including *Zingiber officinalis* (Ginger), *Piper nigrum* (Black Pepper), and *Syzygium aromaticum* (Clove). This finding underscores the high quality and purity of the raw ingredients, reflecting rigorous sourcing, handling, and processing protocols. Adherence to such standards ensures

that the formulation meets pharmaceutical-grade quality, free from contaminants or extraneous substances, which is critical for maintaining its safety and therapeutic efficacy.[27]

pH Values

The pH values of the raw materials spanned a wide range, from 3.54 for *Terminalia chebula* (Haritaki), indicating strong acidity, to 9.35 for *Cinnamomum camphora* (Camphor), reflecting mild alkalinity. Most ingredients, such as *Zingiber officinalis* (4.72) and *Piper nigrum* (5.5), exhibited slightly acidic pH levels. This slight acidity is advantageous in oral care formulations, as it supports antimicrobial efficacy by creating an unfavorable environment for microbial growth while remaining gentle on oral tissues. The balanced pH of the ingredients enhances the formulation's stability and therapeutic potential.[28]

Total Ash Content

The total ash content varied significantly, highlighting the diverse mineral compositions of the raw materials. *Cyperus rotundus* (Nut Grass) exhibited the highest organic ash content (6.82%), indicating a rich mineral profile beneficial for oral health. In contrast, *Acacia catechu* (Black Catechu) had the lowest value (1.41%), suggesting minimal inorganic residues. As expected, *Khatika*, a mineral-based substance, demonstrated the highest total ash content (98.89%). These variations reflect the unique contributions of each ingredient, with higher ash content supporting structural integrity and therapeutic properties, and lower ash content indicating a predominance of organic, bioactive compounds.[29]

Loss on Drying

The moisture content of the raw materials ranged from 4% to 5%, with *Zingiber officinalis* exhibiting the highest value (5.42%). These moderate moisture levels ensure the stability and shelf life of the mouthwash by minimizing the risk of microbial growth or spoilage. Properly controlled moisture content is essential for maintaining the efficacy and quality of the formulation, making these results pivotal for product standardization and long-term usability.[30]

Acid-Insoluble Ash

Most ingredients exhibited low acid-insoluble ash content, with *Acacia catechu* showing the lowest value (0.01%). This indicates minimal non-organic impurities, such as siliceous matter, in the raw materials. Low acid-insoluble ash content is a marker of good quality and

purity, confirming that the raw ingredients meet stringent pharmaceutical standards and are suitable for use in the mouthwash formulation.[31]

Alcohol-Soluble Extract

The alcohol-soluble extractive values highlighted the richness of bioactive compounds in the raw materials. *Terminalia chebula* (44.73%) and *Areca catechu* (42.50%) demonstrated the highest levels, indicating a substantial presence of tannins, alkaloids, and flavonoids, which contribute to the antimicrobial and therapeutic properties of the formulation. *Cinnamomum camphora* displayed complete solubility in alcohol (100%), underscoring its high bioavailability and potential to deliver active constituents effectively. These findings validate the formulation's ability to harness the therapeutic properties of alcohol-soluble compounds for oral health.[32]

Water-Soluble Extract

The water-soluble extractive values revealed a high concentration of bioactive constituents in *Terminalia chebula* (62.29%) and *Areca catechu* (38.25%). These water-soluble compounds, including tannins, glycosides, and phenolic compounds, are critical for the formulation's antioxidant, cleansing, and antimicrobial properties. The presence of such constituents enhances the mouthwash's effectiveness in promoting oral hygiene and preventing microbial infections.[33]

Thin Layer Chromatography (TLC)

TLC analysis provided insights into the chemical diversity of the raw materials by identifying distinct Rf values for each ingredient. For instance:

- *Piper nigrum* displayed multiple Rf values (0.08, 0.52, 0.27), indicative of a complex mixture of bioactive compounds such as piperine.
- *Terminalia chebula* exhibited Rf values of 0.30, 0.54, and 0.62, consistent with the presence of tannins and phenolic acids.

These findings validate the presence of therapeutic constituents essential for the formulation's efficacy and support its use in oral health applications.[34]

Absorbance

Spectral analysis revealed higher absorbance in extracts labeled with a prime (e.g., Sample 1'), suggesting a greater concentration of active compounds in these samples. Absorbance peaks were observed in the 260–300 nm range, which corresponds to the presence of bioactive components such as phenolic compounds and flavonoids. This analysis highlights the chemical richness and therapeutic potential of the extracts, supporting their role in enhancing the efficacy of the formulation.[35]

Antimicrobial Activity

The antimicrobial efficacy of the formulation was assessed against four bacterial strains: *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The results revealed significant variations among batches:

- **Batch 6** exhibited the strongest activity, with inhibition zones of 8 mm against *B. subtilis*, 12 mm against *P. aeruginosa*, 7 mm against *E. coli*, and 25 mm against *S. aureus*. This broad-spectrum efficacy underscores its potential as an effective oral care product.
- **Batch 5** also showed potent antimicrobial properties, particularly against *P. aeruginosa* (17 mm) and *S. aureus* (23 mm).
- Batches 1-4 displayed moderate to no activity, with *Batch 3* showing no measurable effects.

The results of the study demonstrated that **Batch 6** had the strongest antimicrobial activity across all microorganisms, making it the most effective formulation. **Batch 5** also showed significant antimicrobial effects, though slightly less than Batch 6. The other batches (1-4) displayed varying but generally weaker antimicrobial effects, with **Batch 3** showing no activity.

The analysis of the raw materials confirmed their purity, quality, and therapeutic potential. The slightly acidic pH of most ingredients supported their antimicrobial properties, while the total ash and acid-insoluble ash contents provided insight into the mineral and impurity levels. Both alcohol- and water-soluble extracts highlighted the high concentrations of bioactive compounds, particularly in *Terminalia chebula* and *Areca catechu*, which are key contributors to the mouthwash's efficacy.[36]

Safety and Stability

Heavy metal analysis confirmed that the levels of lead, cadmium, mercury, and arsenic were below detectable limits, ensuring the product's safety. Microbial contamination tests revealed minimal bacterial and fungal counts, with no presence of harmful pathogens such as *E. coli*, *Salmonella*, or *Pseudomonas aeruginosa*. These results confirm that the formulation meets stringent safety standards, making it suitable for regular use.

MODE OF ACTION OF DRUGS

Sunthi (*Zingiber officinalis*) Sunthi, with its *Katu* (pungent) *Rasa* and *Ushna Veerya* (hot potency), is known for its anti-inflammatory and antimicrobial effects. Its *Laghu* (light) and *Snigdha* (unctuous) *Guna* enhance circulation in the gums, promoting healing. The *Madhura Vipaka* (sweet post-digestive effect) further aids in balancing the doshas and reducing inflammation, making it an essential ingredient for maintaining oral hygiene.[37]

Haritaki (*Terminalia chebula*) Haritaki, characterized by its *Kashaya* (astringent) *Rasa*, is a potent antioxidant and detoxifier. Its *Laghu* (light) and *Ruksha* (dry) *Guna* help cleanse and tighten the gums, reducing bleeding and inflammation. The *Ushna Veerya* (hot potency) supports circulation, while its *Madhura Vipaka* aids in healing and restoring oral tissue health. Haritaki is particularly effective in treating gum diseases and maintaining oral hygiene.[37]

Musta (*Cyperus rotundus*) Musta has *Tikta* (bitter) and *Katu* (pungent) *Rasa*, with *Sheeta Veerya* (cool potency), making it soothing and anti-inflammatory. Its *Laghu* (light) and *Ruksha* (dry) qualities help balance *Pitta dosha*, reducing inflammation in the gums. The *Katu Vipaka* (pungent post-digestive effect) enhances detoxification, making Musta beneficial in managing oral infections.[39]

Khadira (*Acacia catechu*) Khadira, with its *Kashaya* (astringent) *Rasa* and *Sheeta Veerya* (cool potency), is astringent and antimicrobial. Its *Laghu* (light) and *Ruksha* (dry) *Guna* tighten the gums and reduce microbial load. The *Katu Vipaka* ensures detoxification, while its astringent properties help in reducing gum bleeding and strengthening the teeth.[40]

Karpur (*Cinnamomum camphora*) Karpur has *Tikta* (bitter) and *Katu* (pungent) *Rasa*, with a cooling (*Sheeta Veerya*) effect. It is antiseptic and analgesic, offering relief from pain and reducing infections. The *Katu Vipaka* aids in clearing toxins, while its refreshing properties contribute to oral cleanliness and a pleasant aroma.[41]

Supari (*Areca catechu*) Supari, with its *Kashaya* (astringent) *Rasa* and *Ushna Veerya* (hot potency), is known for its antioxidant and astringent properties. Its *Guru* (heavy) and *Ruksha* (dry) *Guna* tighten gums and reduce plaque accumulation. The *Katu Vipaka* enhances its detoxifying effects, making it a key ingredient for strengthening oral tissues.[42]

Kalimarich (*Piper nigrum*) Kalimarich, with its *Katu* (pungent) *Rasa*, *Laghu* (light) and *Tikshna* (sharp) *Guna*, and *Ushna Veerya* (hot potency), acts as a stimulant and antibacterial agent. It promotes circulation, aiding in faster healing of oral tissues. Its *Katu Vipaka* ensures effective detoxification, while its properties help manage microbial load.[43]

Lavanga (*Syzygium aromaticum*) Lavanga, known for its *Tikta* (bitter) and *Katu* (pungent) *Rasa*, has *Ushna Veerya* (hot potency) that provides analgesic and antimicrobial effects. Its *Laghu* (light) and *Snigdha* (unctuous) *Guna* make it soothing and refreshing. The *Katu Vipaka* enhances its detoxifying properties, making Lavanga effective in reducing pain and promoting oral health.[44]

Dalchini (*Cinnamomum zeylanicum*) Dalchini has *Madhura* (sweet) and *Katu* (pungent) *Rasa*, with *Ushna Veerya* (hot potency). Its *Laghu* (light) and *Snigdha* (unctuous) *Guna* provide antimicrobial and aromatic benefits. The *Katu Vipaka* aids in detoxification, while its properties support gum health and enhance the formulation's flavor.[45]

Khatika Churna (Chalk Powder) Khatika is inert in taste (*Rasa*) and post-digestive effect (*Vipaka*), with *Sheeta Veerya* (cool potency). Its *Guru* (heavy) and *Ruksha* (dry) *Guna* make it effective as a mild abrasive, helping to remove plaque and stains. It also neutralizes acids in the mouth, protecting enamel and preventing decay.[46]

RESULTS AND FINDINGS OF THE STUDY

1. **Foreign Matter** The raw materials used in the formulation, including *Zingiber officinalis* (Ginger), *Piper nigrum* (Black Pepper), and *Syzygium aromaticum* (Clove), were found to be free from foreign matter (0.00%). This confirms the high purity and quality of the raw materials, ensuring the absence of contaminants and compliance with pharmaceutical standards.
2. **pH Analysis** The pH values of the raw materials ranged from strongly acidic (*Terminalia chebula*, pH 3.54) to mildly alkaline (*Cinnamomum camphora*, pH 9.35).

Most raw materials exhibited a slightly acidic profile, which supports antimicrobial activity and suitability for oral care applications.

3. **Total Ash Content** Total ash content varied among the raw materials, reflecting differences in mineral composition. *Cyperus rotundus* (Nut Grass) showed the highest organic ash content (6.82%), while *Acacia catechu* (Black Catechu) had the lowest (1.41%). As expected, *Khatika* exhibited the highest overall ash content (98.89%) due to its mineral-based nature.
4. **Loss on Drying** The moisture content ranged from 4% to 5%, with *Zingiber officinalis* having the highest value (5.42%). This moderate moisture level ensures the stability of the mouthwash and minimizes the risk of microbial growth during storage.
5. **Acid-Insoluble Ash** Acid-insoluble ash was minimal in most raw materials, with *Acacia catechu* showing the lowest content (0.01%). This indicates minimal contamination by non-organic impurities, confirming the quality of the raw materials.
6. **Alcohol-Soluble Extract** The alcohol-soluble extractive values highlighted the concentration of bioactive compounds. *Terminalia chebula* (44.73%) and *Areca catechu* (42.50%) showed the highest values, while *Cinnamomum camphora* exhibited 100% alcohol solubility, indicating complete dissolvability in alcohol. These results confirm the presence of potent bioactive constituents critical for therapeutic efficacy.
7. **Water-Soluble Extract** Water-soluble extractive values were highest in *Terminalia chebula* (62.29%) and *Areca catechu* (38.25%), indicating a rich presence of water-soluble bioactive compounds. These contribute to the mouthwash's antioxidant and cleansing properties.
8. **Thin Layer Chromatography (TLC)** TLC analysis revealed distinct Retention Factor (Rf) values for each raw material, confirming the presence of unique bioactive compounds. *Piper nigrum*, for example, showed multiple Rf values, reflecting its complex chemical profile. These findings validate the therapeutic potential of the formulation.
9. **Absorbance Analysis** The spectral analysis revealed high absorbance in specific samples, particularly those labeled with a prime ('), indicating a greater concentration

of active compounds. Peak absorbance was observed in the 260–300 nm range, highlighting the presence of bioactive constituents.

10. **Antimicrobial Activity**

    - **Batch 5**: Exhibited significant antimicrobial activity with inhibition zones of 17 mm against *Pseudomonas aeruginosa* and 23 mm against *Staphylococcus aureus*.
    - **Batch 6**: Demonstrated the strongest antimicrobial activity, including inhibition zones of 12 mm against *Pseudomonas aeruginosa* and 25 mm against *Staphylococcus aureus*. This batch showed broad-spectrum efficacy.
    - Other batches showed varying degrees of activity, with Batch 3 showing no measurable effects.

11. **Heavy Metals, Pesticide Residue and Microbial Contamination** All batches were free from harmful levels of heavy metals, including lead, cadmium, mercury, and arsenic, the pesticide residue analysis across all batches undetectable levels and did not show any contamination by harmful bacteria such as *Escherichia coli* or *Salmonella*. Fungal contamination was minimal, ensuring the safety of the product.

OUTCOME OF RESULT

Primary Outcome

The primary outcome of the study was to evaluate the **efficacy, safety, and quality** of the mouthwash formulated from *Dashansanskar Churna*:

1. **Antimicrobial Activity**:
    - Batch 6 exhibited the strongest antimicrobial activity, with inhibition zones up to 25 mm (*Staphylococcus aureus*) and broad-spectrum efficacy.
    - Batch 5 also showed significant antimicrobial effects, confirming the mouthwash's effectiveness in oral hygiene.
2. **Safety Profile**:
    - Heavy metal levels were within permissible limits, with no cadmium, mercury, or arsenic detected.

- Microbial contamination was minimal, and no harmful pathogens were present, ensuring the product's safety.

3. **Therapeutic Potential**:
    - Alcohol- and water-soluble extractive analyses revealed high levels of bioactive compounds like alkaloids, phenols, and flavonoids, enhancing antimicrobial and therapeutic properties.

Secondary Outcome

The secondary outcomes assessed the **stability, consistency, and organoleptic properties** of the raw materials and formulation:

1. **Organoleptic Properties**:
    - All batches exhibited a pleasant aroma, slightly irritant taste, and consistent liquid form.
2. **Chemical and Physical Properties**:
    - pH levels (3.47–4.83) were suitable for oral health, supporting antimicrobial action.
    - Specific gravity, refractive index, and viscosity indicated formulation stability.
3. **Batch Consistency**:
    - HPTLC and TLC confirmed the uniform presence of active ingredients across batches, ensuring consistent quality.

These results confirm that the mouthwash is effective, safe, and reliable for oral care.

CONCLUSION

The analytical and antimicrobial evaluation of the raw materials and the mouthwash formulation prepared from the ingredients of *Dashansanskar Churna* provides comprehensive evidence of its safety, efficacy, and quality. The absence of foreign matter across all raw materials confirmed their purity and adherence to pharmaceutical standards. The pH analysis revealed an acidic to mildly alkaline profile for the raw materials, supporting the formulation’s suitability for oral health applications by enhancing antimicrobial efficacy and maintaining oral hygiene. The total ash and acid-insoluble ash content reflected the

mineral and impurity levels, ensuring the ingredients' high quality. Moisture content, as determined by loss on drying, was within acceptable limits, ensuring the formulation's stability and extended shelf life. The alcohol- and water-soluble extractive values underscored the richness of bioactive compounds, particularly in *Terminalia chebula*, *Areca catechu*, and *Cinnamomum camphora*, contributing significantly to the therapeutic efficacy of the mouthwash. Thin Layer Chromatography (TLC) analysis validated the presence of distinct bioactive compounds in each raw material, confirming their role in the formulation's therapeutic action. The absorbance spectra further highlighted the chemical richness of the samples, correlating with their efficacy. The antimicrobial activity demonstrated that Batches 5 and 6 of the mouthwash exhibited the most potent effects against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, emphasizing their broad-spectrum efficacy and potential as effective oral care formulations. the results of this study substantiate the high quality, safety, and therapeutic potential of the mouthwash prepared from the ingredients of *Dashansanskar Churna*. The consistent analytical parameters, combined with significant antimicrobial activity, demonstrate that the formulation meets the standards required for effective Ayurvedic oral health care. This research highlights the importance of rigorous analytical evaluation in the development of traditional formulations, ensuring their relevance and reliability in modern healthcare.

CONFLICT OFI NTEREST –NIL

SOURCE OF SUPPORT –NONE

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