



WORLD JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

www.wjcmpr.com

ISSN: 2582-0222

Development, standardization and microbiological appraisal of herbal dentifrice prepared from *achyranthes aspera* Linn (apamarga) leaves

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Abstract

Dentifrices are the products which are important in our daily life and mainly used to maintain good oral hygiene. The oral hygiene can be maintained throughout the day by using a variety of dentifrices prepared by herbal and synthetic ingredients. Most of the synthetic preparation of dentifrices causes side effects. In this study an attempt is made to prepare a tooth powder which can be used as a tool for proper oral hygiene and to overcome the side effects of synthetic one. The tooth powder was developed using various herbal ingredients such as Apamarga, Clove, Triphala, Pacha karpooora, Ritha and Mulethi. Developed formulation was standardized by analysing necessary evaluation parameters such as organoleptic, microscopical, physicochemical, rheological, phytochemical characteristics. The formulation was screened for its antimicrobial activity by agar well diffusion method against *Streptococcus aureus*. The results showed that the developed dentifrice has promising antimicrobial effect to use against dental diseases.

Article History:



Received: 21.09.2022

Revised: 04.10.2022

Accepted: 24.10.2022

Keywords:

Achyranthes aspera Linn., Standardization, Anti-microbial screening, Dentifrice.

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DOI: <https://doi.org/10.37022/wjcmpr.v4i5.237>

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Introduction

With the prevalence of oral cancer and other conditions rising in emerging nations, oral illnesses continue to be a major global health issue. Oral health is also reflected to body only sporadic success. Alternative prevention and treatment methods need to be secure and efficient health. When it comes to the prevention and treatment of oral disorders, modern medicine has had on a global scale. The basic goal of Ayurveda is the preservation and development of good health; treating ailments is just a secondary goal. An excellent alternative, Ayurveda has the potential to inspire the creation of brand-new preventive and therapeutic methods for dental health. This 5000-year-old medical method not only advises using particular herbs and minerals to treat various dental disorders but also suggests certain regular treatment practices for the preservation of oral health [1].

Dental caries occurs when plaque builds up on the tooth's surface and reacts with the free sugars (all sugars that have been added to foods by the manufacturer, cook or consumer, as well as sugars that are naturally present in honey, syrups and fruit juices) to produce acids that gradually erode the tooth. A persistently high consumption of free sugars, insufficient fluoride exposure and a failure to remove plaque from the teeth with tooth brushing can result in caries, pain and occasionally tooth loss and infection. The gums and bone supporting the teeth are both impacted by periodontal disease.

Pain, bleeding or swollen gums (gingivitis) and occasionally poor breath are the disease's hallmarks. There are more than one billion cases of severe periodontal diseases worldwide, which is believed to affect 14% of adult population. Tobacco smoking and poor dental hygiene are the main causes of periodontal disease [2].

Tooth powder is the product which is used to maintain the oral hygiene such as freshness of mouth and avoid dental disorders. An attempt is made to formulate herbal tooth powder by using ingredients such as Apamarga, Clove, Triphala, Pacha Karpooora, Ritha and Mulethi.

The Apamarga plant leaves have been used as a herbal dental agent for toothache. The fresh leaf is squeezed and its juice is applied to the pain area to get relief. The Apamarga plant is found during monsoon season but rarely seen in other seasons. So, the leaves when available collected, dried, powdered and stored in a suitable container and is applied directly onto the tooth to get relief from toothache. Additionally possessing laxatives, anthelmintics, diuretics, antifungals, antibacterials, anti-allergics, expectorants, stomach tonics and hypoglycemic [3].

Clove is utilized in a number of dental creams, tooth pastes, mouth washes and throat sprays since it is recognized to have antimicrobial characteristics. Additionally, it eases gum discomfort and enhances general oral health. In dentistry, eugenol and zinc oxide are used to temporarily fill cavities.

For dental emergency, Clove is an anodyne (a substance that dulls or relieves pain). Due to the large amount of flavonoids in Clove, it is utilized as an anti-inflammatory [4].

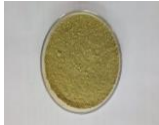
Triphala composed of the three myrobalans *Terminalia chebula* Retz. (Haritaki), *Terminalia bellerica* Roxb. (Bibhitaki) and *Emblia officinalis* Gaertn. (Amalaki). It is an Ayurvedic preparation has been used since ancient times in periodontal therapy. Haritaki treats conditions including typhoid, ascites, biliousness, inflammation, bleeding piles, asthma, sore throat, thirst, vomiting, eye, heart and bladder disorders, as well as constipation, anemia, elephantiasis and delirium. The unripe fruit has astringent properties and is helpful for diarrhoea and dysentery. The Bibhitaki bark is helpful for leucoderma and asthma. The fruit is used for bronchitis, sore throats, biliousness, inflammation and diseases of the eye, nose, heart and urinary bladder. It is also digestible, laxative and anthelmintic. The Amalaki fruits can be utilised as a source of extra vitamin C and other nutrients in medicine [5].

Ritha (Soapnut) is used as a foaming agent. Soapnut is a natural exfoliator and is used medicinally. It possesses insecticidal, anti-fungal and antibacterial properties. It is used to treat coughing and asthma. It works well in Ayurvedic shampoos and cleansers. Psoriasis and eczema are treated with Soapnuts [6].

Pacha Karpooora (Camphor) anti-inflammatory qualities assist it to treat gum disease and toothaches. It aids in easing tooth discomfort and swelling. Additionally, it stops bacteria from spreading to the teeth and gums. Because camphor water has anti-fungal and antibacterial characteristics, it is used to treat skin infections. It assists in controlling lice infestation, dandruff and itchy scalp. In addition, it is used to treat obesity, indigestion and coughs [7].

Mulethi (Liquorice) acts as anti-inflammatory, anti-adhesive and antibacterial characteristics are employed in medicine. Dental caries, gingivitis, periodontitis, aphthous ulcers, and oral cancer can all be helped by it [8].

Dentifrice can be prepared by herbal and synthetic ingredients. Now a days herbal formulations are high in demand and require due to its efficiency to avoid the side effects when compared with synthetic formulations.

Sl.no	Ingredients	Botanical names and family	Plant parts	Photos of sample used	Uses
1	Apamarga	<i>Achyranthus aspera</i> (Amaranthaceae)	Leaves		Dental carries, Anti-inflammatory, Antimicrobial
2	Clove	<i>Eugenia caryophyllus</i> (Myrtaceae)	Flower bud		Analgesic, Anti-inflammatory
3	Triphala (Haritaki, Bibhitaki, Amalaki)	<i>Emblia officinalis</i> (<i>Terminalia chebula</i> Retz., <i>Terminalia bellerica</i> Roxb., <i>Emblia officinalis</i> Gaertn.)	Fruit		Anti-inflammatory, Antibacterial, Analgesic
4	Pacha Karpooora (Camphor)	<i>Cinnamomum camphora</i> (Lauraceae)	White crystalline powder		Anti-inflammatory, Toothache, Flavouring agent, Antimicrobial
5	Ritha (Soapnut)	<i>Sapindus mukorossi</i> (Sapindaceae)	Fruit		Foaming agent, Antimicrobial
6	Mulethi (Liquorice)	<i>Gcyrrhiza glabra</i> (Fabaceae)	Root		Antimicrobial, Dental carries, Anti-inflammatory, Oral cancer

Materials and methods

1) Collection and Identification of herbal ingredients

The ingredients used in the tooth powder are Apamarga, Clove, Triphala, Pacha Karpoora, Ritha, and Mulethi were purchased from local market. Samples were identified in Department of Pharmacognosy at Karavali College of Pharmacy, Mangaluru.

2) Formulation of Tooth powder

Table 01: Composition of tooth powder.

Sl.no	Ingredients	Quantities
1	Apamarga	15g
2	Clove	9g
3	Triphala (Haritaki, Bibhitaki, Amalaki)	2g
4	Pacha Karpoora	0.5g
5	Ritha (Soapnut)	1.5g
6	Mulethi (Liquorice)	2g

Procedure for preparation of tooth powder

The dried powder samples of all ingredients were passed through sieve no.22. Weighed ingredients were triturated using mortar and pestle. Then stored in air tight container in cool and dry place.

3) Evaluation of tooth powder

• Pharmacognostic evaluation

Organoleptic evaluation

In the developed formulation organoleptic properties were studied to determine its colour, odour, taste and texture.

Microscopical evaluation

Formulated product was studied for various microscopical characters.

• Physicochemical evaluation

Prepared formulation was evaluated for various physicochemical parameters such as particle size, moisture content, pH, foamability, spreadability, stability study, extractive values, and ash values.

Determination of particle size

Particle size is a parameter which affect various properties of formulation. Particle size was determined by optical micrometric method [12].

Determination of moisture content

Weighed about 1.5g of the formulation into a weighed flat and thin porcelain dish. Dried in the oven at 100°C or 105°C till constant weight is obtained. Cooled in a desiccator and the loss in weight is recorded as moisture Content [9].

Determination of pH

The pH of 1% solution of formulated tooth powder was determined using digital pH meter [10].

Determination of foamability

About 2.5g of sample was weighed and placed in a 100ml glass beaker 20ml of water added and the beaker was covered with watch glass and allowed to stand for 30min. This operation was carried out to disperse the tooth powder in water. The contents

of the beaker were stirred with glass rod and transferred to a 50ml graduated measuring cylinder during this transfer ensure that no foam was produced. The residue left in the beaker was transferred with 4-5ml of water to the cylinder, stir the contents of the cylinder with the glass rod to ensure a uniform suspension and 12 complete shakes were given to it. The cylinder was allowed to stand for 5min and volume of foam with water and water only was noted for samples.

$$\text{Foaming power} = V1 - V2$$

V1 = volume of foam with water in ml

V2 = initial volume with water

Determination of spreadability

About 0.6g of the sample was weighed and placed at the center of the glass plate and another glass plate was placed over it carefully. Above the glass plates, 1.3kg weight was placed at the center of the plate to avoid sliding of the plate. The sample diameter (in centimetre) was measured after 30 minutes. The experiment was repeated three times and the averages were reported [11].

Stability study

The product was maintained in different temperature conditions to check its stability [13].

Abrasiveness

It is the measurement of the powder fineness that by rubbing on the teeth surface scrubs out the adhered particles of consumed food articles and maintains the shiny smooth surface of teeth. It was measured by rubbing the known amount of each powder on glass slide for 15 minutes with the help of fingertip in the similar manner of brushing the teeth. The scratches on the surface of the slide generated by rubbing the powder were noted down. The results were expressed arbitrarily in positive and negative signs indicating the scratches on glass slide. More positive signs indicated the more abrasiveness [14].

Determination of extractive values

Extractive values are particularly well suited for judging adulteration. It helps to determine the quality as well purity of the product. It also gives an idea about the nature and approximate amount of the chemical constituents present.

Water soluble extractive value: Macerate 5g of formulation with 100ml of chloroform water in two separate closed flasks for 24hours, shaking frequently during first 6 hours and allowed to stand for another 18 hours. Filtered rapidly, taking precautions against loss of chloroform water. 25ml of the filtrate was evaporated to dryness in a shallow tarred flat bottom dish. Dried at 105°C and weighed. Calculated the percentage of water soluble extract with reference to shade dried drug.

Alcohol soluble extractive value : Macerate 5g of formulation with 100ml of alcohol(90%) in two separate closed flasks for 24hours, shaking frequently during first 6 hours and allowed to stand for another 18 hours. Filtered quickly to prevent alcohol loss. 25ml of the filtrate was evaporated to dryness in a tarred shallow flat bottom dish. Dried at 105°C and weighed. Calculated the percentage of alcohol soluble extractive value with reference to shade dried drug [15].

Determination of Ash values

The residue remaining after complete incineration is known as ash content. Ash value is criterion to judge the identity or purity of crude drug.

Total Ash values

Useful for detecting low grade product, exhausted product, excess of sandy and earthy matter with drug.

Accurately weigh 3 g of powdered drug into a tared silica crucible. Gradually increase the heat to burn the powdered medicine and cool it until the free carbon. Store in a desiccator. Weigh the ash and calculate the percentage of total ash for the air-dried sample.

Acid Insoluble Ash values

Used to determine the soil components present in roots, rhizomes and leaves. Boil the total ash obtained above with 25 ml of dill for 5 minutes. hydrochloric acid. Filter and collect insolubles with ashless filter paper. The filter paper is washed with hot water, ignited in a tared crucible, cooled and stored in a desiccator. Calculate the percentage of Acid insoluble ash value with reference to the air-dried drug [16].

Water Soluble Ash values: Used to determine if a material is dehydrated. Boil total ash in 25 ml of water for 5 minutes. The insoluble matter is collected in a gooch crucible or ashless filter paper, washed with hot water and annealed at low temperature to constant weight. Subtract the weight of insoluble matter from the weight of the ash; the difference in weight represents the water- soluble ash. Calculate the percentage of water- soluble ash with reference to the air-dried drug [18].

4) Determination of powder flow property

It gives an overall idea about the viscoelastic flow behaviour of the product. Physical parameters like Angle of repose, Tapped density, Bulk Density, Hausner's ratio, Carr's index and Bulkiness

Bulk density

It is the weight of a volume unit of powder. It is usually expressed in g/cm³, kg/m³ or g/100ml. 25g of weighed powder was taken and slowly poured into the graduated cylinder (100ml). The volume occupied by the powder was given in the formula for calculating the bulk density.

$$D = M/V$$

D = Bulk density

M = Mass of particles

V = Total volume occupied

Tapped density

Tapped density is the increase in bulk density achieved after mechanically tapping a container containing a powder sample. The tapped density is very popular to measure for powder characterization. 25g weighed formulation was taken and slowly added to the graduated cylinder (100ml) with the aid of funnel. After that initial volume was noted and the sample is then tapped until no further volume reduction occurred. The value obtained after tapping was noted. Continued tapping until no further change in volume was observed.

Tapped density = Weight of powder(g)/ Tapped density(ml)

Angle of repose

Angle of repose is the maximum possible angle between the surface of pile of powder and the horizontal plane.

The angle of repose is used as an indicator of the flow ability of bulk solids such as powders or granulate.

The angle of repose of the powder mixture was measured by the funnel method. The accurately weighed powder blend was taken in the funnel. The height(H) of the funnel was adjusted in such a way the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on the surface. The diameter (d) of the powder cone was measured and angle of repose was calculated using the equation.

$$A = \tan^{-1} H/R$$

A = angle of repose

H and R = Height and Radius of the powder cone

Hausner's ratio

Hausner's ratio is the ratio of tapped density to bulk density. It provides an index of flow character of a powder. Hausner's ratio = Tapped density/ Bulk density

Carr's index: It is an indicator of the compressibility of a powder. Carr's index (%) = (Tapped density – Bulk density)/ Bulk density * 100

Bulkiness: Inverse of bulk density.

This is an important consideration when packaging of powders.

Bulkiness = 1/ Bulk density[15]

5) Preliminary phytochemical tests

Aqueous and alcoholic extract of developed formulation was tested for various constituents.

Test for Carbohydrates

Molisch's test: To 2-3ml of extracts, added few drops of Molisch's reagent shake and added conc.H₂SO₄ from sides of the test tube. A violet ring was formed at the junction of two liquids.

Fehling's test: Mixed 1ml of Fehling's A and 1ml of Fehling's B solutions, boiled for one minute. Added equal volume of test solution. Heated for 10-15 minutes in a simmering hot water bath. First a yellow, then brick red precipitate was observed.

Test for Alkaloids

Mayer's test: 2-3 ml of extracts treated with few drops Mayer's reagent, gives creamy white precipitate.

Hager's test: 2-3 ml of extracts treated with few drops Hager's reagent gives yellow precipitate.

Test for Proteins and Amino acids

Biuret test: To 3ml of extracts 1ml of 4% NaOH and few drops of 1% CuSO₄ solution added. Violet or pink colour appears.

Xantho-protein test

3ml of extracts mixed with 1ml conc.H₂SO₄. White precipitate turns yellow on boiling.

Test for Tannins

Ferric chloride test

About 0.5g of the extracts were boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Lead acetate test

To the 1ml of extracts, 1ml of lead acetate solution added. It gives a creamy gelatinous precipitate.

Test for Resins

Acetone water test

Treat extracts with acetone and added to water. Turbidity appears it indicates that presence of resins.

Test for Glycosides (Steroidal glycosides):

Baljet test: The extracts showed yellow to orange colour with sodium picrate.

Borntrager's test (Anthraquinone): To 3ml extracts added dil.H₂SO₄. Boil and filter to the cold filtrate added equal volume benzene or chloroform, shake well separate the organic solvent. Adding ammonia turns the ammonia layer pink or red.

Test for Cyanogenic glycosides**Grignard test**

Soak the filter paper strip first in 10% picric acid then in 10% sodium carbonate, dried. In conical flask place extracts the above filter paper strip is placed the slit in cork. The filter paper turns brick red or maroon.

Test for Coumarin

Alkali test: Extracts when made alkaline, shows blue or green fluorescence.

Test for Saponin**Foam test**

Shake drug extracts or dry powder vigorously with water. Persistent foam observed.

Test for Flavonoids**Shinoda test**

Extracts were treated with magnesium turnings and conc. HCl. Formation of magenta colour.

Lead acetate test

Mix extracts with lead acetate solution. Formation of yellow precipitate.

Test for Lipid

Spot test or Filter paper test: Press the extracts between filter paper. Formation of permanent oily stain [9].

6) Antimicrobial screening

Antimicrobial activity of medicinal plant extracts against the bacterial pathogens prominent in dental carries. It provides adequate protection against microorganism, biological fluids, and aerosols as disease transmission. The antimicrobial assay was conducted using the agar well diffusion method. 1ml of

Streptococcus aureus was mixed into tryptone soya broth before being solidified. The mixture was then transferred into petri dish and plates were dried for 15min and wells were punched using sterile cork borers. 40µl of sample and blank were filled in the well. The plates were incubated for 24 hours at 37°C. After incubation confluent bacterial growth was observed. Inhibition of bacterial growth was measured in mm. A distinct zone of inhibition around the disc indicated the presence of antimicrobial activity [17].

Results and Discussions

Fig 01: Formulated herbal dentifrice

7) Identification of herbal tooth powder

The first step towards ensuring quality of starting material is identification. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity.

The organoleptic and microscopical parameters were observed for all herbal ingredients.

In this study the herbal tooth powder was formulated by using Apamarga, Clove, Triphala, Pacha karpooora, Ritha and Mulethi.

8) Evaluation of herbal tooth powder**Organoleptic evaluation**

The organoleptic studies were performed to identify the colour, odour, taste and texture of herbal tooth powder. Results are shown in table 2.

Table 02: Organoleptic properties

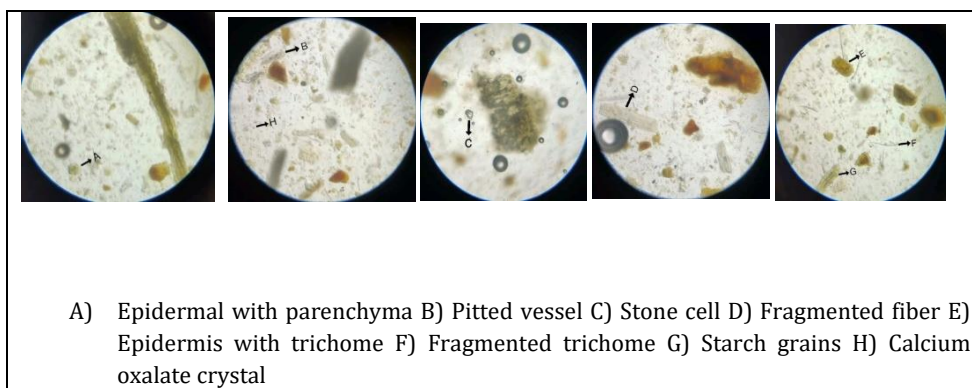
Sl.no	Organoleptic properties	Observations
1	colour	Greenish brown
2	Odour	Aromatic
3	Taste	Sweet and spicy
4	Texture	Fine powder

Table 03: Physicochemical parameters

Sl.no	Physicochemical parameters	Observations		
1	Particle size	150.346µ		
2	Moisture content	14%		
3	pH	4.75		
4	Foamability	4ml		
5	Spreadability	4.7cm		
6	Abrasiveness	++		
7	Extractive values	Alcohol extract	Aqueous extract	
		14%	13.6%	
8	Ash values	Total ash	Acid soluble ash	Water soluble ash
		7.21%	0.91%	2.28%

Microscopical evaluation

Various powder microscopical characters were identified. Results are shown in fig.2

Fig 02: Microscopical characters**➤ Physicochemical evaluation**

The physicochemical evaluation performed to know particle size, moisture content, pH, Foamability, Spreadability, Abrasiveness, Extractive values, and Ash values. Results are shown in table 3.

Particle size is for understanding the physical and chemical properties of a material. The moisture content values of the herbal powder was found to be 14% V/W. It should be in the range of lower than 10% V/W. Below the value of 10% herbal powder will be stable and above the value of 10% will be unstable and there will be chances of microbial degradation. It can be controlled by proper drying of powder and stored in air tight container and kept in dry place.

pH value of tooth powder found to be 4.75. At good pH value it kill the bacteria, remove the risk of cavities, gum diseases and tooth decay.

Foamability of herbal tooth powder was showed 4ml and spreadability of tooth powder was showed 4.7cm. In this study we come to know that developed formulation was easily spreadable and which help to clean the teeth.

Stability studies showed that the product is stable.

Abrasiveness parameter was performed to measure fineness of powder. More positive sign indicates more abrasiveness.

The extractive values of the herbal powder were used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed samples.

The ash values are to remove the traces of organic matter. Results are shown in table 3.

9) Preliminary phytochemical evaluation

The phytochemical studies were conducted based on qualitative analysis to identity the presence of bioactive chemical constituents. Results are shown in table 4.

Table 04: Phytochemical studies

Sl.no	Phytoconstituents	Test	Results	
			Aqueous extract	Alcohol extract
1	Carbohydrates	Molisch's test Fehling's test	+	+
2	Alkaloids	Hager's test Mayer's test	-	+
3	Proteins and amino acids	Biuret test Xanthoprotein test	-	-
4	Tannins	Ferric chloride test Lead acetate test	-	-
5	Resins	Acetone water test	-	-
6	Cardiac glycosides	Baljet test	+	+
7	Anthraquinone glycosides	Borntrager's test	+	+
8	Cyanogenetic glycoside	Grignard test	-	-
9	Coumarin	Alkali test	-	-
10	Saponin	Foam test	+	-
11	Flavonoids	Shinoda test Lead acetate test	-	+
12	Lipid	Spot test	-	-

10) Rheological evaluation

The rheological evaluation was conducted to measure bulk density, tapped density, Angle of repose, Hausner's ratio, Carr's index, and Bulkiness.

Table 05: Powder flow property

Sl.no	Powder flow properties	Observations
1	Bulk density	0.27g/ml
2	Tapped density	0.39g/ml
3	Angle of repose	43.8°
4	Hausner's ratio	1.44g/ml
5	Carr's index	44.44%
6	Bulkiness	3.70

In this study flow property of powder will be poor. By increasing the particle size improves the flow properties due to reduction in the cohesive force. Spherical particles have better flow ability. Drying of particles will reduce the cohesiveness and increase foam.

11) Antimicrobial screening

The zone of inhibition of formulated tooth powder No.2 was found to be 15mm against *Streptococcus aureus*.

Antimicrobial activity test indicates that product inhibits the growth of bacteria, prevent the formation of microbial colonies and may destroy microorganism. By antimicrobial test we concluded that the prepared product is effective against the microorganism present in oral cavity.

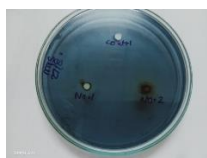


Fig 03: Antimicrobial activity against *S. aureus*

Conclusion

We prepared herbal dentifrice (tooth powder) using plant materials such as Apamarga, Clove, Triphala, Pacha Karpooora, Ritha and Mulethi. Any herbal tooth powder is considered safe to use twice a day hygiene and it does not cause any harmful side effects, instead imparts good freshness and away from bad odour. Oral hygiene can be maintained in a reliable, safe and inexpensive way by using herbal tooth powder. The research concluded that herbal tooth powder an emphasizing and more acceptable in dental research and they are safe. The formulated tooth powder capable to the tooth and oral hygiene and shows the anti-microbial activity against pathogens. The formulated tooth powder has been scope in future in nature of public.

Conflict of Interest

Authors have declared that no conflict of interest exists.

Funding Support

None

Acknowledgement

The authors are thankful to the Department of Pharmacognosy of Karavali College of Pharmacy, Mangaluru, Karnataka, India for providing laboratory facility to carry out the work.

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