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NOVEL FORMULATION OF AYURVEDIC ORAL CARE POWDER AGAINST THE DENTAL CAVITY CAUSING PATHOGEN

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ABSTRACT:

Oral disease is considered one of the major health problems in the world. Dental caries and periodontal diseases are important among these. *Streptococcus mitis* and *Streptococcus mutans* are responsible for dental caries. Many of the market-available oral care powders use chemicals that may cause side effects. The current study aims to formulate the oral care powder using ayurvedic herbs such as *Solanum virginianum* (kantakathiri), clove, bay leaf, cardamom, peppermint leaf, lemon peel, kadukkai. One of the attempts of this study is the use of pumpkin seeds and *Solanum virginianum* (kantakathiri). The methanol, ethanol, and aqueous preparation of this formulated oral care powder is carried out to obtain the highest quantity of active compounds from plants. Evaluation of parameters for the methanol, ethanol, and aqueous was carried out that include an organoleptic and phytochemical evaluation. Phytochemical evaluation is carried out to detect flavonoids, tannins, alkaloids, etc. The anti-microbial activity of the formulated oral care powder is determined using an agar well diffusion assay against the *Streptococcus mitis* isolated from patients with dental caries and the zone of inhibition is measured. The preparation of oral care powder from ayurvedic herbs has a long shelf life, environmentally friendly, and chemical-free method without any side effects.

Keywords: Dental caries, herbal, oral care powder, *Streptococcus mitis, Solanum virginianum,* antimicrobial activity

I. INTRODUCTION: Oral disease is considered one of the major health problems in the world. Dental caries and periodontal diseases are the most important among these. Quality of life is related to oral health and it is also essential for overall health. Dental caries is a chronic disease that destroys enamel. It is one of the infections that can cause periodontitis and gingivitis (Shah, S.T., *et al.*, 2016). The term ‘caries’ originated from the Latin word, which means ‘decay’. Dental caries is not life-threatening, if left untreated it may lead to teeth loss. Dental caries mainly depends upon the oral hygiene of a person (Rathee M, Sapra A. 2023). The major causes of dental caries are fermentable sugars, oral microbial flora, and environmental factors. The acid-producing microorganisms are responsible for dental plaque. Among these, *Streptococcus mutans* and

Streptococcus mitis form lactic acid and other acids and cause dental caries (Dr R. Divya *et al*., 2020). *S. mutans, S. mitis, S. constellatus, S. sanguis, S. salivarius, S. anginosus, S. gordonii, S. intermedius and S. oralis* are the major Streptococcal species that are associated with dental caries. It can also cause the breakdown of dental tissues.

The application of fluoride to teeth is another way to prevent caries that will inhibit the growth of bacteria and also remineralize the tooth surface to prevent the acid attack (Lee, Yoon 2013). Medicinal plants play a major role in the treatment of dental caries. It is the only remedy that does not cause any side effects to us. Even though chemical drugs can inhibit the microorganisms and treat dental caries fast, but the side effects are more (Khan I, and A. Khan 2018). Ayurvedic herbs are proven to be safe and effective. India is rich in a wide variety of plants. Each and every part of the plant is used to treat various diseases. The phytochemical constituents present in the plants show antibacterial, anti-fungal and anti-inflammatory properties that are very helpful for the treatment of diseases.

The stem, roots, and leaves of the plants are used for tooth cleaning and also used to make oral care products. Many studies prove that plants show high antibacterial activity against oral microbial flora (Saini, Rajiv & Sharma *et al*., 2011). The formulation of oral care powder from ayurvedic herbs includes clove, lemon peel, cardamom, peppermint, yellow berried nightshade (kantakathiri), kadukkai, bay leaf, pumpkin seeds, and salt. These herbs had many medicinal properties. The clove which is used as a spice had antibacterial properties. It helps to relieve tooth pain and also inhibits the growth of microorganisms in the dental cavity. *Terminalia chebula* which is commonly known as kadukkai contains tannins. It helps to reduce the growth of *S. mutans.* (Khan I, and A. Khan 2018). The phytochemicals present in the bay leaf are tannins, flavonoids and essential oils. Eugenol is an essential oil which shows an antibacterial property. Lemon is a citrus fruit which is rich in vitamin C. The vitamin C present in the lemon peel is good for oral health and has antibacterial activity against *S. aureus.* It also acts as a tooth-whitening agent. (Annisa, Mutiara & Kanina 2022). Cardamom contains flavonoids, volatile oils, calcium, iron and phosphorus. It has anti-cariogenic properties and also acts as a mouth freshener (Fotedar, Shailee *et al*., 2014). Peppermint acts as a mouth freshener and is also used for tooth whitening. The leaves contain antibacterial, antifungal, and antioxidant properties (Fayed, Marwa, 2019).Kantakathiri is good for oral health and it relieves tooth pain. Pumpkin seeds contain magnesium and phosphorous which is good for teeth.

II. MATERIALS AND METHODS:

1) Isolation and identification of *Streptococcus mitis*

The pure culture of *Streptococcus mitis* isolated on blood agar from patients with dental caries was bought from PSG Institute of Medical Science and Research at Peelamedu, Coimbatore. The pure culture of *Streptococcus mitis* was subcultured on Robertson cooked meat medium (storage medium). The isolates were identified for morphology using Gram staining and biochemical characteristics such as catalase test, oxidase test and methyl red test etc. were performed.

2) Preparation of various extracts of oral care powder

Various extracts of oral care powder such as ethanol, methanol, and distilled water were prepared. 2.5g of formulated oral care powder was added to 25 ml of these solvents in a conical flask and covered it using aluminium foil. Then it is placed in a shaker for 72 hours. After 72 hours, the extract was filtered using filter paper. The filtered extract is then poured on petri plates for evaporation and stored at 4ºC (Mirpour, Mirsasan et al.,2015).

3) Evaluation of parameters

Organoleptic evaluation: The organoleptic evaluation was carried out to determine the colour, odour, taste and texture of the formulated oral care powder.

Phytochemical evaluation: Phytochemical evaluation of ethanol, methanol and aqueous extract of formulated oral care powder was carried out to determine the presence of alkaloids, flavonoids, sterols, proteins, carbohydrates, tannins, cardiac glycosides, anthraquinone, saponins, terpenoids, phenolic compounds, quinones, glycosides, lignin, coumarins and volatile oils.

Detection of alkaloids

Mayer's test: To 2 ml of the filtrate, 1-2 drops of Mayer's reagent were added along the sides of the test tube. The formation of a cream-coloured precipitate indicates the presence of alkaloids.

Detection of anthraquinones

Borntrager's test: 10 ml of 10% ammonia solution was added to 2 ml of the filtrate and shaken for 30 seconds. The formation of reddish-orange colour indicates the presence of anthraquinones.

Detection of carbohydrates

Molish's test: To 2ml of the filtrate, 2 drops of alcoholic α-naphthol and 1ml of conc.H_2SO_4 were added along the sides of the test tube. The formation of a violet ring indicates the presence of carbohydrates.

Fehling's test: 1ml of Fehling's solution A and B were added to 1ml of filtrate and boiled in a water bath. The formation of a red precipitate indicates the presence of carbohydrates.

Detection of cardiac glycosides

Baljet test: To 2ml of the extract, a drop of baljet's reagent was added. The formation of yellow-orange colour indicates the presence of cardiac glycosides.

Keller – Killani test: To 1ml of the filtrate, 1.5ml of glacial acetic acid, 1 drop of 5% ferric chloride and conc.H_2SO_4 were added. The formation of a brown ring indicates the presence of cardiac glycosides.

Detection of flavonoids

Alkaline test: To 1ml of the extract, 2ml of 2% NaOH solution and a few drops of dil. HCl was added. The intense yellow colour with NaOH solution becomes colourless on adding dil. HCl indicates the presence of flavonoids.

Conc.H_2SO_4 test: To 1ml of the extract, 2 drops of conc.H_2SO_4 were added. The formation of reddish-orange colour indicates the presence of flavonoids.

Lead acetate test: To 1ml of the extract, a few drops of 10% lead acetate solution were added. The formation of a yellow precipitate indicates the presence of flavonoids.

Detection of protein

Xanthoproteic test: Few drops of conc. HNO_3 was added to 1 ml of the filtrate. The formation of yellow colour indicates the presence of proteins.

Detection of sterols

Libermann test: To 2ml of the extract, $CHCl_3$, acetic anhydride and conc.H_2SO_4 were added. The formation of a reddish-brown ring indicates the presence of sterols.

Detection of saponins

Foam test: 2ml of water was added to 2ml of extract. The formation of a thick layer of foam indicates the presence of saponins.

Detection of tannins

Braymer's test: To 1ml of the filtrate,3ml of distilled water and 3 drops of 10% ferric chloride solution was added. The formation of bluish-green colour indicates the presence of tannins.

Gelatin test: 2 ml of the extract was dissolved in 5 ml of distilled water. Then add 1% gelatin solution and 10% NaCl to it. The formation of a white precipitate indicates the presence of tannins.

10% NaOH test: 0.4 ml of extract was added to 4 ml of NaOH solution and shaken well. The formation of emulsion indicates the presence of tannins.

Detection of phenolic compounds

Ferric chloride test: Add a few drops of 5% ferric chloride solution to 1 ml of extract. The formation of dark green or bluish-black colour indicates the presence of phenolic compounds.

Gelatin test: 2ml of the extract is dissolved in 5ml of distilled water and add 1% gelatin solution and 10% NaCl to it. The formation of a white precipitate indicates the presence of phenolic compounds.

Detection of glycosides

Borntrager's test: To 2ml of the filtrate, add 3ml chloroform. Shake well and separate the chloroform layer. Then add 10% ammonia solution to it. The formation of the pink-coloured solution indicates the presence of glycosides.

Detection of lignin

Labat test: To 1ml of the extract, add 1ml of gallic acid. The formation of olive-green colour indicates the presence of lignin.

Detection of coumarins

NaOH paper test: 0.5g of the extract is taken in a test tube and the mouth of the test tube is covered with 1N NaOH-treated filter paper. Then heat it for a few minutes in a water bath for the formation of yellow fluorescence which indicates the presence of coumarins.

Detection of quinones

Conc. HCl test: To 1 ml of the plant extract, add conc. HCl. The formation of a yellow precipitate indicates the presence of quinones.

Detection of anthocyanin

HCl test: To 2ml of the extract, add 2ml of 2N HCl and ammonia. The formation of the pink-red solution turns to bluish–violet after the addition of ammonia indicates the presence of anthocyanin.

Detection of terpenoids

Libermann test: To 2ml of the extract, $CHCl_3$, acetic anhydride and conc.H_2SO_4 were added. The formation of a green colour indicates the presence of terpenoids.

Detection of volatile oils

Fluorescence test: 10 ml of extract was filtered till saturation and exposed to UV light. The appearance of pinkish fluorescence indicates the presence of volatile oils (Shaikh, Junaid& Patil, Matsyagandha 2020).

4) Antibacterial activity of formulated oral care powder against *S. mitis*

The antibacterial activity of ethanol, methanol and aqueous extract of formulated oral care powder was carried out using the agar well diffusion method.

Agar well diffusion method: Muller – Hinton agar was prepared and sterilized at 121ºC for 15 minutes. The sterilized media was poured into sterile Petri plates and allow for solidification. After solidification, 2µg/ml of the culture was poured on the media using a micropipette and spread over the entire surface of the plates using an L rod, and allowed for drying. Wells were made in the agar plates using a cork borer. The ethanol, methanol, and aqueous extract of oral care powder were added to the wells. Then the plates were incubated at 37ºC for 24 hours and the zone of inhibition was measured. The antibacterial activity of antibiotics such as gentamycin, ciprofloxacin and azithromycin was also determined for a comparison (Balouiri, Mounyr et al.,2016).

III. RESULTS AND DISCUSSION

1) Isolation and identification of *Streptococcus mitis*

The sample from patients with dental caries was inoculated on blood agar.

Fig 1. Culture of *S. mitis*

Gram staining: In gram staining, gram-positive cocci were observed under a microscope in purple colour. It appears grape-like clusters.

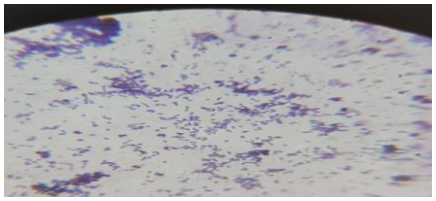

Fig2.Gram staining

Fig 3. Catalase test

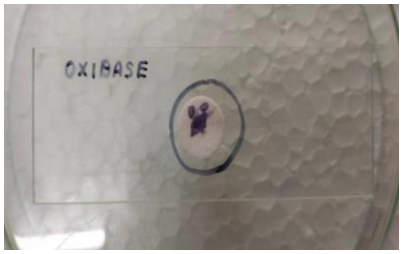

Fig 4. Oxidase test

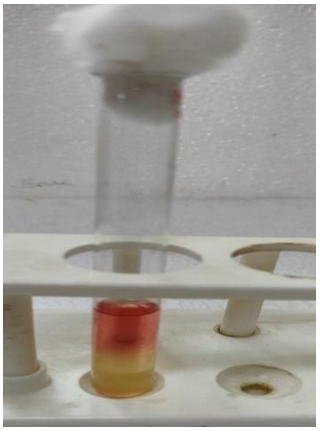

Fig 5. Methyl red test

2) Formulation of oral care powder

Fig no 6. Formulated tooth powder

Table 1. Composition of oral care powder

Sl.no	Ingredients	Quantities
1	Clove	10g
2	Kantakathiri	7g
3	Kadukkai	4g
4	Bay leaf	2.5g
5	Cardamom	2g
6	Lemon peel	1.5g
7	Peppermint	2g
8	Rock salt	2g
9	Pumpkin seeds	4g

Table 2. Biochemical tests

Sl .no	Biochemical tests	Results
1	Catalase test	-
2	Oxidase test	+
3	Methyl red test	+
4	Voges proskauer test	-
5	Citrate test	-
6	Coagulation test	-
7	Carbohydrate fermentation test	-
8	Nitrate reduction test	-
9	Urease test	-

3) Organoleptic evaluation and phytochemical analysis

Table 3. Organoleptic properties

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

Table 4. Phytochemical analysis

Metabolites	Methanol	Ethanol	Aqueous
Alkaloids	+	+	-
Flavonoids	+	+	+
Sterols	+	-	-
Terpenoids	-	+	-
Anthacyanin	-	+	-
Phenolic compounds	-	+	-

Tannins	+	+	-
Cardiac Glycosides	+	+	-
Volatile oils	+	+	-
Saponins	-	-	+
Carbohydrates	+	-	+
Proteins	+	-	+

4) Antibacterial activity solvent extracts of oral care powder against *S. mitis*

Table 5. zone of inhibition

Solvents	**Zone of inhibition**	**Gentamycin**	**Ciprofloxacin**	**Azithromycin**
Aqueous	**15mm**	**15mm**	**20mm**	**25mm**
Ethanol	**10mm**			
Methanol	**50 mm**			

The antibacterial activity of methanol extracts of oral care powder is more than that of antibiotics.

IV. CONCLUSION

The present study shows that the formulated oral care powder from ayurvedic herbs can be used to cure dental caries. The organism *Streptococcus mitis* was inhibited by oral care powder. The antibacterial activity was evaluated using an agar well diffusion assay. The methanol extract of oral care powder forms a high zone of inhibition of a diameter of 50mm compared to ethanol and aqueous extract and other antibiotics. The aqueous and ethanol extract form zone of inhibition of diameter 15mm and 10mm respectively. The study concluded that oral care powder from ayurvedic herbs is also more effective than commercially available dentifrices.

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