



Formulation And Evaluation of Antibacterial Polyherbal Dentifrices

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Abstract :-

Dentifrices have been used since the ancient past and one of main irreplaceable components of oral health care. During that period, squashed bone, pulverized egg and clam shells were utilized as abrasives as a part of tooth cleaning. In recent years, focus has shifted towards the release of active ingredients during formulation developments to prevent and /or treat oral illness. But some substances show unpleasant side effects such as tooth staining and altered taste. This led to increased attention of using natural ingredients in herbal dentifrices. Herbal ingredients have several benefits. Commonly used herbal ingredients are Neem (*azadirachta indica*) and Guava+ (*Psidium guajava*). Neem have antibacterial and anti-inflammatory properties. Neem also have anti caries properties. Dried chewing twigs of neem shows maximum antibacterial activity against *S. mutans*. Polyherbal dentifrices was prepared using Neem, acacia, guava, calcium carbonate, sodium lauryl sulphate. The formulated polyherbal dentifrices was evaluated for organoleptic and physical properties such as colour, odour, taste, pH and fineness according to standards specified by Bureau of Indian Standards. Antimicrobial susceptibility testing methods was adopted by *disc diffusion* method. Lab made polyherbal dentifrices was formulated by selecting suitable ingredients to get the formulation more stable. The preliminary in vitro study demonstrated that polyherbal dentifrices was found to be efficacious in terms of all evaluation properties of dentifrices. Hence, by the evidence of in vitro studies, it is concluded that polyherbal dentifrices formulated in a laboratory was found to be good antibacterial property and good quality.

Keywords :- Dentifrices, Antimicrobial, Antibacterial, Disc diffusion, Antiinflammatory.

Introduction :-

Dentifrices have been used since the ancient past¹ and are one of main irreplaceable components of oral health care². The design of dentifrices formulations began in China and India, as 300-500 BC. During that period, squashed bone, pulverized egg and clam shells were utilized as abrasives as a part of tooth cleaning³. Modern toothpaste formulations were developed in the 19th century. Later on, chalk and soap were incorporated to those formulations. After 1945, several formulation development of different detergents had begun, sodium lauryl sulfate had been used as emulsifying agent. In recent years, the focus has shifted towards the release of active ingredients during formulation developments to prevent and /or treat oral illness.¹ The majority of the cleaning is performed by the mechanical utilization of the toothbrush with the help of excipients used in dentifrices. The main aim of this investigation is to evaluate the polyherbal dentifrices formulations.

Dentifrice apply with a toothbrush, to clean and keep the aesthetics and health of teeth. Toothpaste serves as an abrasive that aids in removing the dental plaque deposit on teeth is a concern because of its cosmetic and pathogenic nature. Presence of plaque may be the culprit deposit for dental caries, gingivitis, periodontal problem, and halitosis. So many mechanical aids are used in dental worldwide to detach or control plaque, including toothbrushes, dental floss, and mouth rinses, and dentifrices (Barnes VM, Ritcher R, De Vizio W, 2010). Mechanically plaque removal is one of the most trusted methods of controlling plaque and gingivitis. Mechanical plaque control is time consuming and some individuals may lack motivation for these procedures (Mullaly BH et al, 1995).³ Chemical plaque control and thus reduce or prevent oral disease. Several chemicals, preventive agents have favourable effects in controlling plaque and to reduce or prevent oral disease. Hence, various chemical formulations were tried in dentifrices (George J et al, 2009). Chemicals, mainly triclosan and chlorhexidine have been added in mouth rinses and dentifrices to prevent plaque and gingivitis. But these substances show unpleasant side effects such as tooth staining and altered taste (Barnes VM, Ritcher R, De Vizio W, 2010 and De Oliveira SM et al, 2008). This led to increased attention of using natural ingredients in herbal dentifrices. Herbal ingredients have several benefits. Commonly used herbal ingredients are Neem (*azadirachta indica*), etc.

Neem possess antibacterial (Nayak A et al, 2011) and anti-inflammatory properties. Neem also have anti caries properties (Prashant GM et al, 2007 and PackiaLekshmi NCJ et al, 2012). The antimicrobial effects of neem have been described against *S.mutans* and *S.faecalis* (Siswomihardjo W, 2007 and Almas K, 1999). Dried chewing twigs of neem shows maximum antibacterial activity against *S.mutans* (Chava VR et al, 2012). There are limited studies available regarding the efficacy of herbal dentifrices hence the present study was undertaken to assess their effect on oral hygiene and gingival bleeding.³

Natural products have been used for several years in folk medicine (Omar, 2013). Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world (Chitme, 2003). Over the last decennium herbal medications used in both prophylaxis and treatment of various diseases turned to be a favoured form of therapy throughout the world. Many side effects associated with traditional medicines have been averted by using herbal medicines and thus they are safer to use. Some natural products are marketed for oral and dental use to assure the shift to usage of natural products from pharmaceutical products among the public. These alternative products can be either dental products with natural ingredients or herbal products.⁴ Previous studies have revealed that dental plaque can be controlled by physical removal and use of various pharmaceutical formulations like herbal dentifrices, antimicrobial toothpastes and mouthwashes. Mechanical plaque control methods include tooth brushing and interdental cleansing using oral hygiene aids and professional prophylaxis. Chemical plaque control has been used only as adjunct to mechanical means and not a substitute even though various chemicals are widely used nowadays.

Some chemicals, preventive agents have beneficial effects in the control of plaque and to reduce or prevent oral disease. Hence, various chemical formulations were tried in dentifrices.⁵ Chemicals, mainly triclosan and chlorhexidine have been added in mouth rinses and dentifrices to prevent plaque and gingivitis. But some of these substances show undesirable side effects such as tooth staining and altered taste⁶. This led to give attention on using natural ingredients in herbal dentifrices. Herbal ingredients have several benefits. Commonly used herbal ingredients are Neem (*azadirachta indica*), Guava+ (*Psidium guajava*) etc. Neem have antibacterial and anti-inflammatory properties.⁷ Neem also have anti caries properties⁸. The antimicrobial effects of neem have been reported against *S. mutans* and *S. faecalis*⁹. Dried chewing sticks of neem shows maximum antibacterial activity against *S. mutans*¹⁰. There are limited studies available regarding the efficacy of polyherbal dentifrices hence the present study was undertaken to assess their effect on oral hygiene and gingival bleeding.

The objective of the present research work to formulate polyherbal dentifrices containing natural ingredients like Neem leaves, Guava leaves, etc. which were traditionally used for tooth cleaning. In present study formulated polyherbal dentifrices was evaluated for its organoleptic and physical properties such as colour, odour, taste, pH, fineness, etc. as per standards specified by Bureau of Indian Standards.

MATERIALS AND EQUIPMENTS :

Materials

Table no-1

No	Common name	Botanical name	Parts used	Category
1	Neem	<i>Azadirachta indica</i> (Meliaceae)	Leaves & bark	Antibacterial
2	Guava	<i>Psidium guajava</i> (Myrtaceae)	Leaves	Anti-inflammatory
3	Calcium carbonate	—	—	Abrasive
4	Acacia	—	—	Prevention & Treatment of Gingivitis
5	SLS	—	—	Detergent
6	Saccharin sodium	—	—	Sweetening Agent
7	Propyl paraben	—	—	Preservative
8	Amaranth	-	—	Colouring Agent

EQUIPMENTS

Table No-2

Sr No.	Equipments	Model No.
1	Digital pH meter	—
2	Incubator	MVTEX
3	Analytical balance	AUW0D

EXPERIMENTAL DEVELOPMENT :

Procedure of preparation of polyherbal dentifrices:

Polyherbal dentifrices was prepared using Neem, acacia, guava, calcium carbonate, sodium lauryl sulphate. Neem leaves posses anti-bacterial activity. Guava leaves shows an positive activity in case of mouth ulcer. Sodium lauryl sulphate used giving foaming. Propylparaben is preserve the product. Acacia to prevent gingivitis.

All herbal additive were dried and grounded using domestic mixer. The required quantity of ingredients were weighed and taken in mortar. Calcium carbonate, sodium lauryl sulphate, sodium saccharin, amaranth were mixed in mortar. Acacia were added into the above mixture. This mixture containing herbal ingredients and triturated well untill a consistency is formed.

Final batch formula

Table no-3

Ingredients	Quantity (gm)
Neem leaves	3.0
Amrud leaves	5.0
Calcium Carbonate	5.0
Acacia	3.0
Sodium Lauryl Sulphate	2.5
Saccharin sodium	1.0
Propyl paraben	0.5
Amaranth	0.1

Evaluation of herbal dentifrices :

Composition : Dentifrices is not composed of mono or disaccharides such as sucrose or fermentable carbohydrates. All ingreadients should comply with the Indian standards.

Colour :The prepared polyherbal dentifrices was evaluated for its colour.The colour was checked visually.

Odour : Odour was found by smelling the product.

Taste : Taste was checked manually by tasting the product.

Determination of sharp and edge abrasive particles :

Force out the contents 15-20 cm long on the butter paper, repeat the same process for at least ten collapsible tubes. Press the contents of the with finger tip for the presence of sharp and hard edged abrasive particles. Dentifrices shall not contains such particles.

pH determination :

Weigh 10 g of dentifrices placed in 150 ml beaker. Allow 10 ml of boiled and then cooled water. Stir vigorously to make a suspension. Measure the pH of the suspension using pH meter.

In vitro antibacterial activity

Method of antibacterial susceptibility testing:

Antimicrobial susceptibility testing methods was adopted by *disc diffusion* method. The pathogenic organism was grown on Nutrient agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks was an indirect measure of the ability of that compound to inhibit that organism, known as zone of inhibition.

Test microorganisms:

The one bacterial strains were used in the present study which obtained from Department of Microbiology, Institute of Kamla Nehru College of Pharmacy , Butibori, Nagpur University. The bacterial strains used were *Staphylococcus aureus*, The effects of the formulation on the bacterial strains were assayed by disc diffusion method.

Preparation of Nutrient Agar Medium:

2.8 gm of Nutrient agar media (Issued from Microbiology laboratories) was suspended into 100 ml distill water and mixed properly. After that it was heated to dissolve the media completely. Sterilized by autoclaving at 15 Lbs pressure and 121⁰ C temperature for 15 minutes, mixed well before use.

Preparation of Nutrient agar plates:

Approximately 25 ml of liquid Nutrient media was poured (to a depth of 4 mm) into Petri plates and allowed to solidify at room temperature, stored at 4 to 80C temperature, pH of the Nutrient agar should fall between 7.2 and 7.4 at room temperature after solidification.

Reading plates and interpreting results:

After 14 to 16 hours of incubation, each plate was examined. If the plate was adequately streaked, and the inoculum was correct, the resulting zones of inhibition should be uniformly circular and a confluent lawn of growth. The diameters of the zones of absolute inhibition (as judged by the unaided eye) were computed, including the diameter of the disc. Zones were measured to the nearest whole millimeter with the help of a ruler, which was held on the back of the inverted Petri plates. The Petri plate was held a few inches above a black, nonreflecting background and illuminated with reflected light. The zone margin should be taken as the area showing no apparent, visible growth that can be detected with the unaided eye. Faded growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, should be ignored. After measuring the diameter of zone of inhibition the data was noted and interpreting the result.

Antibacterial activity of Polyherbal dentifrices with standard Antibiotic Agent:

Antibacterial activity of dentifrices was performed. The determination of developed herbal composite and standard antibiotic agent (Ciprofloxacin) against dental isolates was assessed through standard well diffusion method. The isolates were inoculated onto Muller-Hinton agar plates using sterile cotton swab. Wells were punctured (6mm) and standard antibiotic agent (Ciprofloxacin) loaded separately with developed polyherbal composite (at a concentration of different concentration). All the loaded plates were incubated at 37 °C for 24h to observe zone of bacterio stasis (mm) around each well against each of the dental isolates.

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RESULT AND DISCUSSION :

Polyherbal dentifrices formulation was prepared from Neem leaves, guava leaves natural ingredient and small amount of synthetic ingredients.

Following results are observed :

Table No-4

Sr. No	Parameters	Observations
1	Colour	Pale greenish
2	Odour	Characteristics
3	Taste	Sweet
4	Abrasiveness	Good abrasive
5	pH	6.97

Table 5-Antimicrobial activity of polyherbal dentifrices against standard(Ciprofloxacin) :

Batches	Zone of Inhibition (in mm) (<i>S. aureus</i>)
D1	20.8mm
D2	20.6mm
S1	40mm
S2	40.1mm

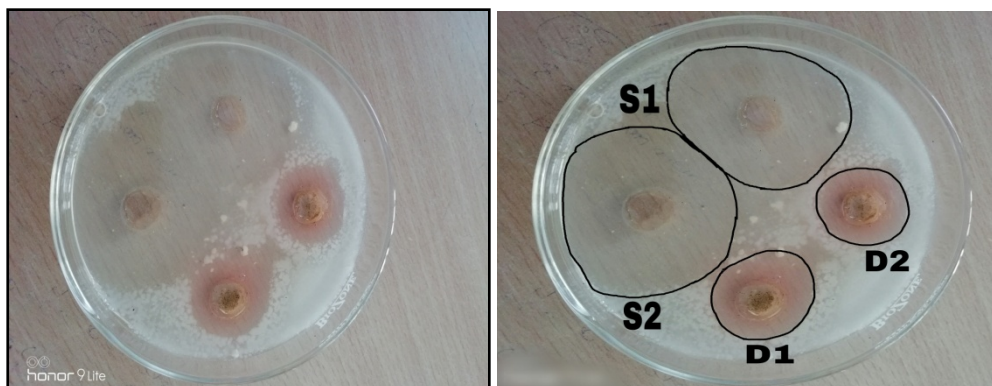


Fig 1: Zone of inhibition-Polyherbal Dentifrices (D1 & D2) compare with Standard (S1 & S2)

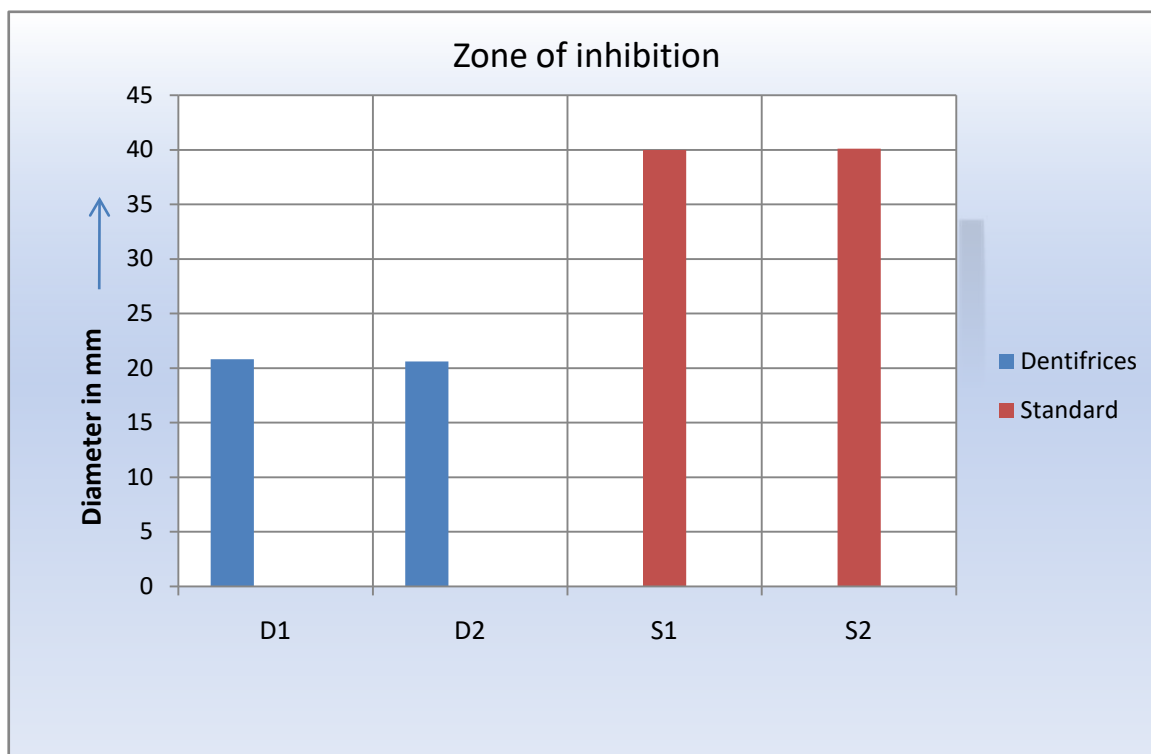


Fig 2 : Zone of Inhibition

CONCLUSION :

Eventually polyherbal dentifrices having an emphasized role in the maintaining the oral hygienic nature as well as preventing dental caries. Based on this pattern, Lab made polyherbal dentifrices was formulated by selecting suitable ingredients to get the formulation more stable. The preliminary in vitro study demonstrated that polyherbal dentifrices was found to be efficacious in terms of all evaluation properties of dentifrices. Hence, by the evidence of in vitro studies, it is concluded that polyherbal dentifrices formulated in a laboratory was found to be good antibacterial property and good quality.

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