

Formulation and Evaluation of a Polyherbal Toothpaste using Medicinal Plants

Urmila Nishad¹, Meraj Ali², Anupama Maurya³

^{1,2}Faculty at Gaya Prasad Institute of Human Excellence for Pharmacy, Malihabad (Lucknow)

³J.P. Verma College of Pharmacy, Hardoi

Abstract

There are 45000 ancient medicinal plant species in India targeted spots within the region of Japanese chain, Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. The formally documented plants with healthful potential area unit 3000 however ancient practitioners use over 6000. India is that the largest producer of healthful herbs and is termed the "Botanical garden of the world". There is unit presently 2, 50,000 registered medical practitioners of the Ayurvedic system. The current study was aimed to formulate and evaluate Polyherbal dentifrice exploitation normally out there healthful plants in Lucknow as to treat oral problems.

The Six plant samples that has been employed in this project i.e., **Neem, Clove, Betel, Peppermint, Turmeric and Guava**, are normally used as ancient medicines. However these plants conjointly contribute a good deal to the ayurvedic medicines since history. They not only show antimicrobial property for naturally action diseases however even have essential concentration of bound phytochemical present in them which will profit our body in some ways. Therefore during this project, **phytochemical screening (qualitative** – saponin, tannin, flavonoid, carbohydrate, protein, alkaloid, phenol, Coumerin, Quinones, terpenoids and soluble starch, and of these six plant samples has been carried out along with the **antibacterial** activity of these plant samples against some chosen bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*)

Oral health could be a part of general health and is very important within the traditional development of the kid. Cavity is that most current dental wellness moving humankind spittle acts as a protecting issue against cavity development by providing the most defense system for the host. Saliva contains great amount of proteins and amino acids that help to maintain the homeostasis of oral cavity.

The developed toothpastes were evaluated as per standards per Bureau of Indian Standards. The antimicrobial potency of the read toothpastes were determined. Antimicrobial study showed that the formulations have important activity against *Staphylococcus aureus*, *Escherichia coli* and Oral micro flora. Polyherbal **dentifrice** containing aqueous extract of Neem, clove, betel, peppermint turmeric and Guava leaves was **developed** and tested for medicinal drug activity against crocus aureus, Bacillus cereus, Escherichia coli and Oral micro flora with different concentrations of **dentifrice** were used (100, **hundred** mg/ml).

Among all the tested **bacterium** used *Staphylococcus aureus* was found to be most sensitive to the **developed dentifrice** as seen by zone of inhibition (6-7mm) followed by *Escherichia coli* (4-6 mm), and *Oral micro flora* (5-8mm). The **developed** Polyherbal **dentifrice** was with success evaluated **exploitation completely** different standard parameters to confirm its quality and physiochemical properties. The results showed that the developed Polyherbal dentifrice is promising antimicrobial effects against each gram positive and gram negative organisms. It should be safer compared to completely artificial dentifrice. More studies are warranted to prove safety and effectuality of the development Polyherbal dentifrice.

The research concluded that Herbal toothpaste an emphasizing and more acceptable in dental research and they are safer with minimum side effect than synthetic preparation. The formulated toothpaste capable to the tooth and oral hygiene show the antimicrobial activity against pathogen. The formulation compared with market preparation. Therefore it shows the equal patronizing and engrossing passion over the marketed formulations (Colgate, Dabour Red, and Dantkanti). The formulated herbal toothpaste has been good scope in future in nature remedies research and Dental health of public.

Keyword: Neem, Clove, Betel, Peppermint, Guava, Turmeric, Toothpaste, Poly Herbal formulation, Antibacterial activity, Evaluation Parameters

INTRODUCTION

India is a one of the most important country to be known for the ancient script the number system invention of zero and Vedas. In India, medicines are used about 60% World's population. These are not used for only primary health care and not just in rural areas in developing countries, but they have also developed countries as well where modern medicines are predominantly used. While traditional medicines are obtained medicinal plants, minerals, and so on herbal medicines of organic matter are prepared medicinal plants only. Drugs have been used as the source of the plants and important component of the health care system in India. The Indian system of Medicine, most practitioners should make and share their own recipes, so it requires proper documentation and

research. In the west, use of herbal medicines is increasing with the use of reporting about 40% of the population within the last one year of medical diseases to treat herbs. Due to the general public, education and government's interest in increasing are traditional medicines increasingly due to side effects of adverse drug reactions and cost-factor modern medical systems. There are approximately 45,000 species of medicinal plants in India with locations centered in the eastern Himalayas, Western Ghats and the areas of Andaman and Nicobar Islands. Through the formally recognized, the medicinal capability of plants is 3000 then use traditional experts and more than 6000. India is the largest producer of medicinal herbs and it is called "Botanical garden" of the world. There are presently around 2, 5,000 listed medicinal experts of the

Ayurvedic system, as associated toward around 7, 00,000 of the new drug system. Depending on the 70% of population in rural India, there is a traditional medicine type Ayurveda. In India, there are many forms of alternative medicines available, those who do not want to do, traditional Medicine or what cannot be helped traditional medicine, Ayurveda and Kabiraji (herbal medicine) are two important forms that are alternative medicine Available widely in India. Ayurvedic medicines can be considered as the form of equivalent to in thousands of years India. It provides various techniques and things for employment to ill patients or patients for relief. One of the things that ayurveda uses medicines of plant origin. In Traditional systems, different indigenous plants are being used physical Mental or social imbalance of diagnosis, prevention and eradication. The drugs are obtained with whole plant of different organs like leaves, stems, barks, root, flowers, seeds, etc. The source of medicinal plants are the active chemical components involved in medicinal plants because synthetic drugs and antibiotics associated with the health hazards and toxicity associated with the increase of human diseases in order to eliminate important therapeutic help, the indiscriminate use of synthetic drugs and antibiotics.

MATERIALS AND METHODS

Chemicals-

Calcium carbonate (Fisher Scientific), Glycerine (Rankem.), Sodium lauryl sulphate (Fisher Scientific), Acacia gum (Rankem), Sodium Chloride (Willson), Sodium Saccharine (Willson), Para hydroxyl benzoic acid (Fisher Scientific) were purchased from the market.

Collection

a. Sample collection

Leaves of neem (*Azadirachta indica*), Peppermint (*Mentha piperita*), Turmeric (*Curcuma longa*), Clove (*Syzygium aromaticum*), Betel (*Piper betle*) and Guava (*Psidium guajava*) sample were collected from local market of Lucknow. The sample were stored at room temperature (37 °C) until further use.

b. Drying

Drying of the leaves of Neem (*Azadirachta indica*), Peppermint (*Mentha piperita*), Turmeric (*Curcuma longa*), Clove (*Syzygium aromaticum*), Betel (*Piper betle*) and Guava (*Psidium guajava*) was done for one week at room temp.

c. Crushing

Crushing of the leaves was done with the help of pestle and mortar at room temperature. The crushed sample was stored at room temp.

d. Aqueous extraction

Extraction is that the crucial start within the analysis of healthful plants, as a result of it is necessary to extract the required chemical elements from the plant materials for any separation and characterization. The fundamental operation enclosed steps, similar to pre-washing, drying of plant materials or freeze drying, grinding to get the same a sample and sometimes homogeneous extraction

humanizing the dynamics of analytic abstraction and conjointly increasing the interaction of sample superficial with the solvent system. Proper actions ought to be taken to assure that potential active constituents don't appear to be lost, distorted or destroyed throughout the preparation of the extract from plant samples.

Formulation of Toothpaste

All herbal ingredients were dried and grounded using domestic mixer. The required quantity of Ingredients were weighed and taken in mortar. Calcium carbonate, Sodium lauryl sulfate, methyl Cellulose, honey and Glycerine were mixed in water. Acacia were added into the above mixture. This solution was added drop wise into mortar containing herbal ingredients and triturated well until a paste consistency is formed. Table 1 shows plant extracts and composition of chemicals.

Table 1: Chemical Composition of Formulation

S.No.	Excipients	Quantity in (gm)		
		F ₁	F ₂	F ₃
1	Neem extract	2	3	1
2	Clove	2	2	2
3	Peppermint	1	2	1
4	Guava	2	3	1
5	Betel	2	3	2
6	Turmeric	1	2	2
7	Calcium Carbonate	20	20	20
8	Glycerine	5	5	5
9	Sodium lauryl sulphate	1	1	1
10	Acacia gum	0.5	0.5	0.5
11	Sodium Chloride	0.5	0.5	0.5
12	Sodium Saccharin	0.5	0.5	0.5
13	Para hydroxide benzoic acid	1	1	1
14	Distilled water	60-80ml	60-80ml	60-80ml

Procedure

3gm of the Neem extract, 2gm of Clove extract, 2gm of Peppermint extract, 3gm of Guava extract, 3gm of Betle extract, 2gm of turmeric extract were triturated with 1gms of Para hydroxyl benzoic acid and 0.5 gm of sodium chloride (as a preservative) in a Mortar-pestle. 1gm of the sodium lauryl sulphate are using as foaming agent and sodium saccharin are added as a sweetener agent. Further 5 ml of Glycerine was added as humectant and acacia gum are used as a binder, triturated well and to adding 80 ml of demineralized water was added to make up the to100gm.

pH is adjusted with a solution of sodium hydroxide. Clove oil is added to mask the bitter taste.

Evaluation of Toothpaste

Physical Examination

- **Colour-** Formulated toothpaste was evaluated for its colour.
- The visually colour was checked.
- **Odour-** Odour was found by smelling the product.
- **Taste-** Taste was checked manually by tasting the formulation
- **Relative density-** Relative density was determine by weight in gram taken in 10 ml formulation and 10 ml distilled water using RD bottle

Evaluation Parameters

- **Abrasiveness-** Extrude the content 15-20 cm long on the butter paper, repeat the same process for at least ten collapsible tubes. Press with the contents of the entire length with fingertip for the presence of sharp and hard edged abrasive particles. Toothpaste shall not contain such particles.

Determination of spreadability

In this method slip and drag characteristic of paste involve. Formulated paste (2g) placed on the ground slide under study. The formulated paste placed like sandwich between this slide and another glass slides for 5min to expel air and to provide a uniform film of the paste between slides. Excess of the paste was scrapped off from the edges. The top plate was then subjected to pull of 80g with the help of string attached to the hook and time (sec) required by the top slide to cover a distance of 7.5cm was noted. A short interval indicated better spreadability.

Formula was used to calculate spreadability:

$$S = M \times L / T$$

Where,

S= Spreadability

M= Weight in the pan (tied to the upper slide)

L= Length moved by the glass slide

T=Time (sec) taken to separate the upper slide from the ground slide.

pH determination

pH of formulated herbal toothpaste was determined by using pH meter. 10g of toothpaste placed in 150ml of beaker. Allow the 10ml of boiled and then cooled water. Stir vigorously to make a suspension.

Homogeneity

The toothpaste shall extrude a homogenous mass from the collapsible tube or any suitable container by applying of normal force at $27 \pm 20^\circ\text{C}$. in addition bulk of contents shall extrude from the crimp of container and then rolled it gradually.

Foaming

The foamability of formulated toothpaste evaluated by taking small amount of formulation with water in measuring cylinder initial volume was noted and then shaken for 10 times. Final volume of foam was noted

Determination of froth power

Foaming power = $V_1 - V_2$

V_1 - Volume in ml of foam with water.

V_2 - Volume in ml of water only.

Stability

The stability study was performed as per ICH guideline. The formulated paste was filled in collapsible tube and stored at different temperature and humidity conditions, $25^\circ\text{C} \pm 2^\circ\text{C} / 60\% \pm 5\% \text{RH}$, $30^\circ\text{C} \pm 2^\circ\text{C} / 65\% \pm 5\% \text{RH}$, $40^\circ\text{C} \pm 2^\circ\text{C} / 75\% \pm 5\% \text{RH}$ for the period of three months and studied for appearance, pH and spreadability.

Determination of moisture and volatile matter

5 g of formulation placed in a porcelain dish containing 6-8 cm in diameter and 2-4 cm depth in it. Dry the sample in an oven at 105°C .

Calculation

By mass = $100\text{MI}/\text{M}$ MI-Loss of mass (g) on drying

M- Mass (g) of the material taken for the test.

PHYTOCHEMICAL TEST

a. SAPONIN

- 2 ml sample was dissolved in 6ml distilled water.
- Shaked well. Froth formation took place.
- Stability of the froth confirms the presence of saponin in the samples.

b. TANNIN

- 1 ml sample was dissolved in 1 ml 5% FeCl_3 .
- Appearance of dark blue or greenish black color confirms presence of tannin the sample.
- If no color changes then heating mantle is used for changing the color.

c. FLAVANOIDS

- 2 μl samples was drop wise added into 20 ml NaOH.
- Again Conc. HCL was added drop wise, appearance of yellow color
- Confirms the presence of flavonoids in the sample.

d. CARBOHYDRATES

- Fehling's reagent was prepared by mixing Fehling A and Fehling B solution.
- For Fehling A- 0.35g CuSO_4 was dissolved into 5 ml distilled water followed by addition of 2-3 drops of Conc. H_2SO_4 .
- For Fehling B- 1.75g NaK tartarate was dissolved in 5 ml distilled water, 1.25g NaOH was added in the solution and mixed well to dissolve it.
- Then Fehling A and Fehling B was mixed well in the ratio of 1:1 (FA+FB=10ml).
- Now 1ml Fehling's reagent was dissolved in 2ml sample and heated for over 20 mins.
- Appearance of red ppt. confirms the presence of carbohydrates in the sample.

e. PROTEIN

- 500µl of 1% CuSO₄ was prepared and 500µl of 5% NaOH was prepared.
- Mixed together.
- Sample was added in the solution, occurrence of purple color confirms protein in the sample.

f. ALKALOIDS

- 500µl extract was centrifuged and 500µl Wagner's reagent was mixed into it.
- Shaked well and left for some time.
- Reddish brown color appears and confirms presence of alkaloids.

g. STARCH SOLUTION

- Add the sample
- Add 2-3 drops of yellow iodine solution
- Stir with glass rod
- The iodine solution will turn blue/black colour then starch is Present.

h. FAT TEST.

- Press the small quantity of extracts between two filter
- Paper the strain on one filter indicated the presence of fixed oils.

i. TERPENOID TEST

- 500µl sample was dissolved in 250µl chloroform.
- 625µl Conc. H₂SO₄ was added to the solution.
- Reddish brown ppt. of the solution confirms presence of terpenoids

j. PHENOL TEST

- 500µl extract was dissolved in distill water. 2 drops of aq.
- FeCl₃ was added.
- Appearance of blue color or green color indicates presence of phenols.

h. COUMERIN TEST

- Take a look at 10% NaOH was additional to the extracts and CHCl₃ was additional for observation.
- Yellow colour that show the presence of Coumerin.

i. QUINONES TEST

- Take a look at dilute 10% NaOH was additional to the 1ml of crude extracts.
- Blue-greenin experienced or red coloration indicated the presence of quinones.

ANTIBACTERIAL ACTIVITY**Aim-**

Anti-microbial test is performed in this case to see the inhibitory effect of the mentioned toothpaste samples.

Growth and Maintenance for Bacteria-

The strains of bacteria *S. aureus*, *E. coli* and *Oral micro flora* was provided by Rapture Biotech for this particular study.

The strain from the plate was inoculated in the nutrient broth and then the inoculum was left for 1-2 days at 37°C in the incubator. After the growth of bacteria in the broth, it is used to perform the well diffusion method with the given sample.

Bacteria used

- *Staphylococcus aureus* (*S. aureus*)
- *Escherichia coli* (*E.coli*)
- *Oral micro flora* (*Oral Bacteria*)

PROCEDURE**Preparation of sample**

The plant extracts are first allowed to dry in a petridish to get its concentrated form.

Before performing the test, the dried plant extracts were added with little amount of distilled water to make a conc. solution.

Preparation of plates

- Weigh allreagents and dissolved in 60 ml water.
- Heated with agitation to dissolve the constituents properly.
- Autoclaved at 121°C and 15 lbs. pressure.
- Immediately after autoclaving, allow it to cool in a 45 - 50°C.
- Pour the freshly prepared and cooled medium into petri plates.
- The agar medium should be allowed to solidify at room temperature.

Spreading of bacteria

- Take a cotton swab that has already put in UV light, and dip it into a freshly prepared culture of *S. aurues*, *e.coli*, and *Oral micro flora*
- Get rid of the extra liquid in the swab and then spread evenly on the surface, of the plate so that a bacterium is spread in each corner of the plate.
- Let it dry for 4-5 minutes.

Incubation

- Incubate the plates in the incubator for overnight at a temperature of 37° C

Reading of plate and interpretation

After 15 to 16 hours of incubation, each plate was examined. If the plate satisfactory streaked, the inoculums were correct the result of ZOI should be uniformly circular and a confluent lawn of growth. After measure the diameter of ZOI the data was noted and interpreting the result.

Comparison: Formulated herbal toothpaste with marketed preparation

The formulated herbal toothpaste was compared with marketed preparation follows Anti-microbial activity, Spreadability, Foamability, pH determination, % Moisture content.

RESULT-

Table.2. Phytochemical Test

Test	Neem	Turmeric	Clove	Peppermint	Betel	Guaava
Alkaloid	+	+	+	+	+	+
Saponin	+	+	+	+	+	-
Tannin	+	+	+	+	+	+
Flavanoid	+	+	+	+	+	-
Protein	-	+	+	-	-	+
Terpenoid	+	-	+	-	+	-
Carbohydrates	+	+	-	-	+	+
Coumerin	-	-	+	-	+	+
Quinones	-	+	+	-	-	-
Starch	+	+	+	-	+	+

Physical Examination

Table.3. Evaluation of herbal toothpaste

S. No.	Parameters	Observation
1	Colour	Mud green
2	Odour	Pleasant
3	Taste	Better
4	Smoothness	Smooth
5	Relative density	10.2

Evaluation Result

S. No.	Parameters	Observation
1	pH determination	7.76
2	Foaming determination	13 ml (good)
3	Moisture content	15.46 %
4	Spreadability	3.5 cm/sec
5	Homogeneity	good
6	Abrasiveness	Good abrasives
7	Stability	Stable
8	Microbial growth	No M

Extrudability

Extrudability	Mean of Three tube
Net wt. of formulation in tube (g)	13.5
wt. of toothpaste extruded (g)	12.2
Extrudability amount percentage	90.32

Stability

At 25°C ±2°C/ 60% ± RH (3rd month)

Colour	Appearance	Spreadability	pH
Mud green	Homogeneous	3.5	7.76

At 35°C ±2°C/ 65% ± RH (3rd month)

Colour	Appearance	Spreadability	pH
Mud green	Homogeneous	3.45	7.25

At 40°C ±2°C/ 75% ± RH (3rd month)

Colour	Appearance	Spreadability	pH
Mud green	Homogeneous	3.0	7.0

The stability study was indicated that the formulated tooth paste was good stability.

Oral Bacteria-

The test organisms were isolated and identified and the results were recorded based on the morphological and gram stain microscopic.

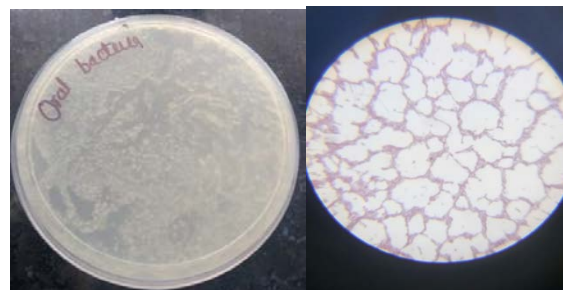


Fig. 1 & 2 Oral Bacteria plate and gram stain result

Anti-Microbial Activity Observation

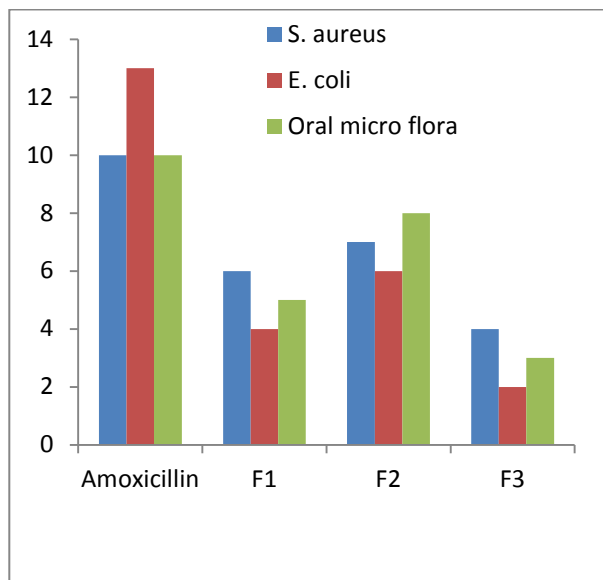
The formulated herbal toothpaste exhibited fairly good anti- *S.aureus* activity as compared to the standard drug Amoxicillin. The formulation exhibited an impressive ZOI of 7 mm at MIC of 25µg/mL, whereas Amoxicillin exhibited 10 mm ZOI at MIC of 6.25µg/mL. Therefore it may be concluded that formulated tooth paste have potential to exhibit anti-microbial activity.



Fig Zone of inhibition of F1, F2 and F3 against 1.Oral bacteria, 2. E.coli, 3. S. aureus

Table 4. Inhibition zone of Toothpaste Formulation

Bacteria	Amoxicillin	F1	F2	F3
S. aureus	10	6	7	4
E. coli	13	4	6	2
Oral micro flora	10	5	8	3

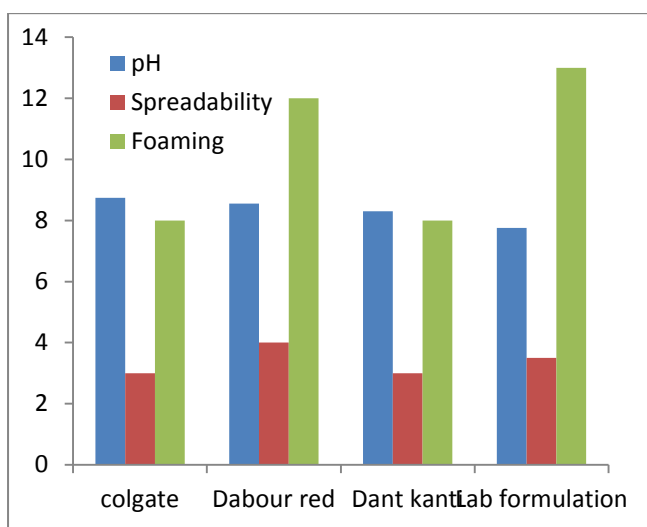


Graph 1. Depicting Antibacterial activity of Toothpaste formulation F1, F2 and F3

Comparative study: Formulated herbal toothpaste with marketed preparation

Table 5. Comparison between pH, Spreadability & Foamability with marketed preparation.

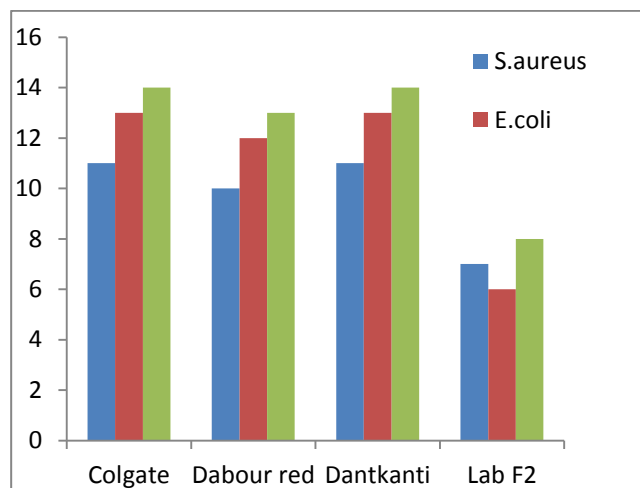
Formulation	pH	Spreadability	Foaming
Colgate	8.74	3	8
Dabour red	8.55	4	12
Dant Kanti	8.3	3	8
Lab formulation	7.76	3.5	13



Graph 2. Comparison between pH, Spreadability & Foamability with marketed preparation

Table 6. Inhibition Zone of marketed formulation Toothpaste

Bacteria	Colgate	Dabour red	Dant kanti	F2
E.coli	11	10	11	6
S. aureus	13	12	13	7
Oral bacteria	14	13	14	8



Graph 3. Comparison of anti-microbial activity with marketed formulation Colgate, Dabour red, Dant kanti and F2

Table 7. % Moisture Content Comparison

S. No	Preparation	% Moisture Content
1	Colgate	15.10
2	Dabour Red	25.15
3	Dant Kanti	10.20
4	Lab Formulation	15.46

The formulated herbal toothpaste is having an equal or near about and engrossing passion over the marketed preparation (Colgate, Dabour Red and Dantkanti).

CONCLUSION

Neem's findings are taken in very small amounts due to bitterness, this bleeding act as anti-inflammatory component against the gums. Mint is known for its aroma hence it helps to get rid of bad breath. Clove is applied on the gums (used topically) for aching, for pain management throughout dental work, and for a complication of tooth extraction known as "dry socket." it's conjointly applied to the skin as a counter pain in the neck for pain and for mouth and throat inflammation. It helps in the destruction of oral microorganisms by preventing oral pathogens such as pyorrhea and cavities. Turmeric is used to replace chloroxidine, an anti-microbial and anti-septic agent that is used in oral hygiene.

Leaves of Neem (*Azadirachta indica*), Peppermint (*Mentha piperita*), Turmeric (*Curcuma longa*), Clove (*Syzygium aromaticum*), Betel (*Piper betle*) and Guava (*Psidium guajava*) leaf were extracted by aqueous method. In the qualitative phytochemical testing presence of assorted secondary metabolites were found in binary compound.

The developed dentifrice was evaluated for chemistry parameters such as color, odour and hydrogen ion concentration.

The development dentifrice was tested for antibacterial drug against *Staphylococcus aureus*, *Escherichia coli* and micro the efficiency was qualitatively and quantitatively assessed by the presence or absence of a zone of inhibition and zone diameter values. The developed dentifrice exhibited extremely vital result towards the entire tested microorganism, whereas the negative management doesn't turn out noticeable repressive result for any of the tested microorganism

Neem (*Azadirachta indica*), Peppermint (*Mentha piperita*), Turmeric (*Curcuma longa*), Clove (*Syzygium aromaticum*), Betel (*Piper betle*) and Guava (*Psidium guajava*) were potential for inhibition bacteria. The antibacterial drug activity of the developed Polyherbal dentifrice conjointly showed vital antibacterial drug activity against all the tested microorganisms. This observation indicates that the activity to the presence of huge kinds of phytoconstituents present within the extract. Hence, the ascertained antibacterial drug activity of the dentifrice was thanks to the presence of active constituents of the extract and therefore activity also well maintained once it absolutely was reborn to dentifrice. This was sensible sign to try to additional studies thereon to create it together of the business flavoring dentifrice for the treatment of oral microorganism infections.

The research concluded that Herbal toothpaste an emphasizing and more acceptable in dental research and they are safer with minimum side effect than synthetic preparation. The formulated toothpaste capable to the toothpaste and oral hygiene show the antimicrobial activity against pathogen. The formulation compared with market preparation. Therefore it shows the equal patronizing and engrossing passion over the marketed formulations (Colgate, Dabour Red, and Dantkanti). The formulated herbal toothpaste has been good scope in future in nature remedies research and Dental health of public.

REFERENCE

- Adejumo OE, et al., Assessment of *in-vitro* and *in-vivo* antimicrobial activities of selected Nigerian toothpastes and mouth washes on some oral pathogens. *Adv. in Med. Dent. Sci.* 2008; 2(3):61-5.
- Anna T, et al., Remineralization potential of a new toothpaste formulation: An *In-vitro* Study. *The J. of Cont. Dent. Practice* 2004; 5(1):1-12.
- Collins L et al., Instant tooth whitening from a silica toothpaste containing blue covarine. *J. of Dentistry* 2006; 36(8):21-5.
- D. Mamatha *IOSR Journal of pharmacy and biological Sciences (IOSR- JPBS)* Volume 12, Issue 6 Ver. V (Nov.-Dec.2017)
- Golpar R, et al., study to assess the plaque inhibitory action of herbal-based toothpaste: A double blind controlled clinical trial. *J. of Medicinal Plants Research* Vol. 4(12), pp. 1182-1186, 18 June, 2010.
- Kandarp Dave *International Journal of chemistry and Pharmaceutical Sciences*, 2014, vol.2 (3): 666-673.
- Kolpo AL, et al., Phytochemical composition and antimicrobial activity of propos is Africana against some selected oral pathogens. *World J. Agric. Sci.* 2009; 5(1): 90-93.
- Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anti-cancer agents. *Fitoterapia.* 2010 Oct; 81(7):703-14. Doi 10.1016/j.fitote.2010.06.004. Epub 2010 Jun.
- Mahendra sekar et al *International journal of pharmaceutical and clinical research* 2016; (8):1155-1158.
- Meyers AL, et al., The surface effect of dentifrices. *Australian Dent J.* 2000; 45 :2):118-124.
- Mridul Haque *Journal of chemical and Pharmaceutical Research*, 2014, 6(3): 1279-1285.
- Nitin CM, et al., Chitosan-based toothpaste: As novel oral hygiene product. *Indian J. Dent Research.*21(3), 2010.
- Okpalugo J, et al., Toothpaste formulation efficacy in reducing oral flora. *Trop. J. of Pharm. Research* 2009;8(1):71-7.
- Ozdemir A, et al., The determination of salivary zinc level following delivery from zinc containing toothpaste. *J. of Med. Sciences* 1998; 28(11):281-3.
- Praphasri R, et al., Effects of fluoride dentifrice on remineralization of demineralized primary enamel. *South East Asian. J. Trop. Med. Public Health.* Vol 41 No. 1 January 2010.
- Ramya kuber *international journal of pharmaceutical and clinical research* 2015.
- Rantamen I, et al., Effects of a betaine containing toothpaste on subjective symptoms of dry mouth: A randomized clinical trial. *The J. of Cont. Dent. Practice.* 2003; 4(2):1-10
- Siddharth Sharma *International journal of pharmaceutical Research & Allied Sciences.* Volume 3,issue 2 (2014), 30-39.
- Stephen YG, Francis A, Marcel TB, Vivian EA, Kofi A. In vitro antimicrobial study of the efficacy of a toothpaste formulated from Granica Kola stem wood. *Int. J. of Pharmacy and Pharm Sci* Vol 2, Issue 2, 2010.
- Stratul SL, et al., Antimicrobial agent in periodontal disease therapy: rationale and use of the triclosan-PVM/MV copolymer in the management of gingivitis. *J. of Dent. Research* 2008; 59(1):105-108.
- T. Mangilal, *International journal of Ayurvedic & herbal medicine* 6 (3) May-June 2016 (2266-2273).
- Vaibhav shende, *European journal of pharmaceutical and medical research*, January 2017.
- Wei Hu, Yan HZ, Wang Q, William D and Margaret P. Effects of fluoride toothpaste on etched enamel of orthodontic patients. *Chin. J. of Dent. Research* 1999; 2(3):79-83.