Formulation And Evaluation of Herbal Toothpaste of Murraya Koenigii Leaf Extract

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Abstract-The objective of this research was to determine the antibacterial activity of herbal toothpaste from the extract of Murraya koenigii leaves on Streptococcus mutans and Escherichia coli. Toothpaste was formulated with different concentrations of Murraya koenigii leaves, F1 extract (25 ppm), F2 (50 ppm), F3 (75 ppm) and F4 (100 ppm) on both aqueous extract by maceration and ethanolic extract Soxhlet extraction. Each formulation was tested the physical characteristics and antibacterial activity toward S. mutans and Escherichia coli to obtain the comparative study on aqueous and ethanolic extract potency of herbal toothpaste. Antibacterial activity was determined by Agar Well Diffusion method using mutans Hinton Mueller agar plates. Also, antibacterial activities were assessed by the presence or absence of inhibition zones after the plates were incubated at 37°C for 24 hours. The results of this test illustrated that all the toothpastes under study exhibited antibacterial activity at different concentrations of Murraya koenigii leaves. Antibacterial activity test showed maximum zone of inhibition by F4 (100 ppm) of ethanolic extract herbal toothpaste against Streptococcus mutans. The ethanolic extract herbal toothpaste shows better antibacterial action when compared to the aqueous extract. Therefore, these toothpastes can be used as natural antibacterial agents in preventing tooth decay caused by S. mutans and Escherichia coli. Toothpaste extracted from Murraya koenigii leaves of ethanolic extract showed significant antibacterial activity against S. mutans and E. coli in high concentration.

Key words: Curry leaves, Murraya koenigii leaf extracts, herbal toothpaste, Comparative study, Streptococcus mutans, Escherichia coli and Barrier zone.

INTRODUCTION

In developing countries, the severity of infections caused by certain pathogenic microorganisms can lead to mortality and morbidity in immuno-suppressed patients.[1]

Many abrasive materials, scented, green lead was used to remove strains from teeth until the mid-19th century. In the Middle Ages, rock salt and fine sand were the main materials used by the Arabs for tooth cleaning. 1950 AD by Dr. Washington Wentworth Sheffield Dentistry.[2]

DEFINITION OF TOOTHPASTE

Toothpaste is defined as a semi-solid dosage form that is used with the aid of toothbrush to remove naturally occurring deposits from the teeth and enhance the oral hygiene.[3]

Toothpastes may also help with plaque control and removal, promoting healthy gums. Regular use of toothpaste helps to avoid dental problems. From 5000 BC the Egyptians used powdered cow's hooves, myrrh, powdered and burnt eggshells, and pumice. Crushed bones and oyster shells were added by the Greeks and later the Romans to improve the formulation of toothpaste. Toothpaste or powder became popular in the nineteenth century. The natural ingredients in the toothpaste provide protection and care to the teeth and dental health.[4]

IDEAL PROPERTIES OF TOOTHPASTE

- Excellent abrasive properties
- Non-irritant and non-toxic
- Provides fresh breath
- Reducing tooth sensitivity

- Reducing gum disease
- Significant reduction in plaque
- Provides germ protection
- Gum bleeding should be reduced
- Good abrasive effect
- Not expensive
- Easily available
- Acceptable taste
- With less side effects
- Keep the mouth clean[5]

Herbal based toothpaste has been used in ancient life since the production and development of toothpaste products starts in China and India 300 to 500 BC and at that time crushed bones, crushed eggs and muscle shells were used as abrasives as part of dental cleanings. Modern toothbrushes were developed in the 19th century After the development of the drug, salt and soap are added to these forms.[6]

Herbal medicines are used by 60% of the world's population. They are used not only for basic health care, but also in rural areas in developing countries. Traditional medicines are derived from medicinal plants, minerals and so on. They are an important part of the health care system in India. Increasing public awareness of traditional medicines which have fewer side effects, adverse drug reactions and lower cost factors as compared to modern medicine.[7]

TYPES OF TOOTHPASTE

Toothpastes are of following type on basis of their uses: -

1. Natural Toothpaste:

They are made from natural ingredients such as essential oils and herbal extract like neem. They are natural alternative of chemical base toothpaste and do not contain fluoride which is highly recommended by dentists due to its enamel protective properties and teeth strengthening.

2. Anti-caries Toothpaste:

They are mainly used for cavity protection. They contain fluoride to stop tooth enamel decalcification and protect teeth from tooth decay and cavities. They contain anti- bacterial agents like sodium fluoride and sodium mono-fluorophosphate.

3. Whitening Toothpaste:

They are used in whitening of teeth. They are useful for anyone who has yellow teeth or those who consume too much tobacco, soft drinks, tea and coffee. They contain abrasives that remove some types of pigmentation and stains, but have a major side effect that they erode the enamel layer present on teeth over time.

4. Sensitivity:

Toothpaste They are used to treat the sensitive teeth. They contain desensitizing agents like potassium nitrate, potassium citrate & strontium chloride, to relief tooth sensitivity by blocking the dentinal tubules, that prevent the nerves in the teeth from feeling hot and cold, and helps in providing relief to people who are suffering from toothache.[8,9,10]

DENTAL HEALTH

Dental health is one of most integral part of our overall health as well as well-being. Poor dental health leads to various dental disorders. Dental disorders are caused by an imbalance in the doshas (mainly kapha), according to Ayurveda. A balanced dosha system in the mouth ensures that all of the doshas' duties assist our overall health.[11]

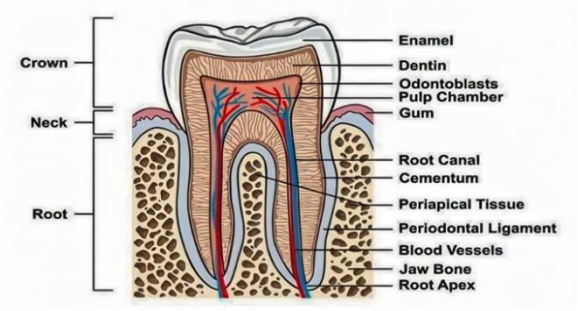


Fig 1: Transverse view of human tooth

The anatomical part of the tooth consists of crown, root, enamel, dentine and pulp. Many problems related to teeth include bad breath, tooth decay, gum disease, tooth sensitivity, calculus, tooth decay and plaque.[12]

Structural components of teeth

- ENAMEL is a hard outer layer consisting primarily of calcium and phosphate in the form of hydroxyapatite.
- DENTIN is the inner layer, the bulk of the tooth.
- PULP is core, containing nerves and blood vessels.
- CEMENTUM is the thin layer around the root; a bone-like material which connects the teeth to the jaw.[13]

MATERIALS AND METHODS

Collection of Murraya koenigii leaves

The fresh leaves of Murraya koenigii was collected from the home garden, medicinal garden and our

college campus in Tiruvannamalai, Tamil Nadu. The leaves of the plant were authenticated by the faculty of Government college of Arts and Science, Tiruvannamalai.

Fig 2: Fresh leaves of Murraya koenigii
Fig 3: Dried curry leaves

Collection of chemical ingredients
The excipients used in the formulation are purchased from the local market and our college laboratory.

Microorganism
S. aureus and E. coli, pathogenic bacteria isolates were acquired from Department of Biotechnology, Shanmuga of Arts and Science College, Tiruvannamalai. The bacteria were culture in Hinder-Muller Agar Media at 37^{O}C for 24 hours.

Preparation of Murraya koenigii leaf extract
The Murraya koenigii leaves were collected and washed with running water for 2-3 times and allow to dry for 10 days. The dried leaves are subjected to size reduction by using the electrical blender. The powder leaf sample are pass through the sieve no. 80. Finally, the Murraya koenigii leaf powder was collected and stored in the air tight container for the further use.

Preparation of aqueous extract of sample

Maceration method:
The dry sample powder was immersed in aqueous solvent (water) and allowed to macerate for six days. Following the macerated time, the extract is filtered through filter paper. The filtrate is collected and stored in an airtight, well-closed container labeled 'Aqueous Extract'.

Preparation of ethanolic extract of sample
Soxhlet method:
Assemble the apparatus properly with stands and clamps. The round bottom flask is put on the heating mantle, and the temperature is kept around 35-40^{O}C. The flask is connected to the Soxhlet extractor and condenser via the water inlet and outflow pipes. The use of cotton clogs the side arm. The crushed Murraya koenigii leaves were loaded into a thimble and placed in the Soxhlet extractor. The extract is heated using the heating mantle. The solvent from the extract was evaporated and passed through the condenser. When the vaporized solvent passes through the condenser, it becomes liquid and settles on the thimble containing the powdered sample. The powdered sample is entirely soaked by the solvent and then falls into the round bottom flask. The process runs for 24 hours at 40°C.after that the extract is filtered through filter paper. The filtrate. is collected and stored in an airtight, well-closed container & labeled.

Fig 4: Soxhlet apparatus for the extraction of Murraya koenigii leaf extract

Simple Distillation
Separation of fresh extract from the solvent was done by simple distillation. This is accomplished by attaching a distillation flask to a side arm that slopes downward. The flask mouth is closed with a cork and

contains a thermometer. For cold water circulation, the inlet and exit are located on the condenser's lower and upper sides, respectively. A liquid product is delivered through the condenser output and collected in the receiver.

PHYTOCHEMICAL INVESTIGATION OF MURRAYA KONEIGII LEAF EXTRACT [14-17]

S.no.	Chemical test	Procedure	Observation	Inference
1.	Alkaloid test	Mix 2 ml of leaf extract, 2 – 3 drops of dil. HCl and 1 ml of Drangendroff's reagent.	The solution turns orange color into red color precipitate	Presence of alkaloid
2.	Carbohydrate test	Add 2 ml of conc. H2SO4 to the 2ml of leaf extract	The interface of the two layers forms red or dull purple color	Presence of carbohydrate
3.	Cardiac glycoside test	Add a few drops of conc. H2SO4, glacial acetic acid and ferric chloride to the leaf extract	It produces blue-green color	Glycoside is present
4.	Amino acid test	Add 2-5 drops of ninhydrin solution to the leaf extract and boiled it in a water bath for 2 minutes	The solution turns into purple color	Presence of amino acid
5.	Terpenoid test	Mix a few drops of chloroform and conc. H2SO4 to the leaf extract	The red-brown precipitate forms	Terpenoid is present
6.	Quinone test	Add a few drops of conc. HCl to the 2 ml of plant leaf extract with vigorous mixing in the test tube	The solution shows yellow precipitate	Presence of quinone
7.	Phenol test	Add 2-3 drops of 1% ferric chloride to the leaf extract stirrer vigorously	The solution produced green/blue/bluish green/ brown/ brownish red color	Presence of phenol
8.	Flavonoid test	Add 3 ml of dil. NH_3 and 1 ml of conc. H_2SO_4 to the leaf extract	The color of the solution turns into yellow color	Flavonoid is present
9.	Saponin test	Shake the leaf extract with 10 ml of distilled water vigorously to form stable froth and add a drops of saturated oil mix thoroughly	The emulsion is formed	Presence of saponin
10.	Anthraquinones test	Boil the leaf extract with 4 ml of conc. H2SO4 and 3ml of chloroform. The chloroform layer was pipette out into another test tube containing diluted ammonia	The solution appears pink red/violet color at lower phase	Presence of anthraquinones.
11.	Reduced sugar test	To the 0.2 ml of powdered plant sample, add 1 ml of ethanol and 2 ml of distilled water in two test tube separately. After that add 1 ml of Fehling's A and B solution in both test tube and boiled it.	The color changes	Presence of reducing sugar
12.	Tannin test	Boil the leaf extract with distilled water and add 0.1% of ferric chloride	The solution produced brownish green or blue or black color	Presence of tannin
13.	Gum test	Add the leaf extract to distilled water in the test tube and shake it vigorously	The solution forms swells or adhesives	Presence of gum
14.	Coumarin test	Add 10% of NaOH and chloroform to the leaf extract.	The solution gives yellow color	Presence of coumarin
15.	Steroid test	Mix a few drops of conc. H2SO4 and leaf extract	The solution appears the green color	Presence of steroid

FORMULATION OF MURRAIYA KONEGII LEAF EXTRACT TOOTHPASTE

Procedure

The herbal toothpaste is made using the dry gum process. The steps for making toothpaste are listed below. To begin, the base is prepared by mixing solid materials such as calcium carbonate, sodium lauryl sulphate, and sodium chloride, which are carefully weighed as specified in the formula. Then, mix the solid materials together thoroughly and screen it through sieve no. 80 to obtain a fine powder of solid

ingredients. After that, pour the fine powder into the mortar and firmly triturate it with a pestle. Add glycerin to the fine powder and continuously triturate with a pestle until it reaches a semi-solid consistency. Finally, combine the Murraya koenigii leaf extract with the semi-solid formulation and aggressively stir in the peppermint oil. The technique was repeated for both the aqueous and ethanolic extracts of Murraya koenigii at concentrations ranging from 25 to 100ppm. The formulated toothpaste was stored in a collapsible tube and labeled.[18]

Composition of herbal toothpaste[23]
All ingredient should be complied with the Indian Standard
Table 1: Composition of herb and chemicals

S. No	INGREDIENTS	QUANTITY	USES
1	Plant extract (ethanol and aqueous)	Various concentration (0.5,1,1.5 and 2%)	Active Ingredient
2	Calcium Carbonate	3.5g	Abrasive Agent
3	Glycerin	2ml	Humectants
4	Sodium lauryl sulphate	0.5g	Foaming Agent
5	Sodium Chloride	0.2g	Thickening agent
6	Methyl paraben	0.3g	Preservative
7	Saccharin	0.3g	Sweetening Agent
8	Pepper mint oil	0.5ml	Flavoring Agent
9	Titanium oxide	0.1g	Coloring Agent

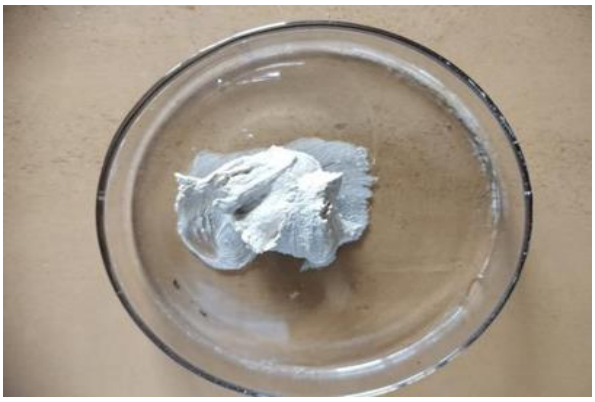


Fig 5: Aqueous extract pastes


Fig 6: Ethanol extract pastes

EVALUATION PARAMETERS OF FORMULATED HERBAL TOOTHPASTE [21 & 24-37]

1. PHYSICAL EXAMINATION

Color - The prepared toothpaste's color was evaluated visually.
Odor - Odor was discovered by sniffing the formulation.
Taste- Taste was assessed manually by tasting the formulation.

2. pH

10g of toothpaste was placed in a 150ml beaker. Add 10ml of hot water and then cooled it. Stir vigorously to form a suspension. The pH value should be recorded using a pH meter.

3. HOMOGENEITY

To extrude toothpaste from a collapsible tube or container, apply normal force at 27 ± 20C.

4. TUBE INERTNESS

The toothpaste container should not corrode or deteriorate under standard storage settings, such as a heating temperature of 45±20C for 10 days. Tube inertness can be determined by cutting the internal surface open and observing for signs of deterioration or chemical attack in the container.

5. DETERMINATION OF SHARP AND EDGE ABRASIVE PARTICLES

Extrude the content 15-20 cm long onto the butter paper; repeat for at least ten collapsible tubes. Press the contents of the entire length with a fingertip to

detect the presence of sharp- and hard-edged abrasive particles. Toothpaste must not include such particles.

6. FOAMABILITY

The foamability of designed toothpaste was examined by adding a tiny amount of formulation to a measuring cylinder having distilled water, noting the initial volume, and shaking 10 times. The final volume of foam was recorded.

7. DETERMINATION OF MOISTURE AND VOLATILE MATTER

5 g of formulation was placed in a porcelain dish about 6-8 cm in diameter and 2-4 cm thickness. Dry the sample in an oven at 105 °C. Calculation % by

mass = 100MI/M

MI-Loss of mass(g) on drying

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

8. EXTRUDABILITY

The prepared paste was filled into a typical capped collapsible aluminum tube and sealed by crimping the end. The weight of the tubes was noted. Clamps were used to hold the tubes between two glass slides. 500g was placed over the slides, and the cover was removed. The amount of extruded paste was collected and weighed. The percentage of extruded paste was calculated.

9. SPREADABILITY

Spreadability was determined using a Spreadability device composed of a wooden board with a scale and two glass slides with two pans on each side fixed on a pulley. Excess sample was sandwiched between two glass slides, and a 100 g weight was applied to the glass slide for 5 minutes to compress it to a uniform thickness. Weight (250 g) was added to the pan. The time in seconds necessary to separate the two slides was used to assess 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.

10. STABILITY STUDY

The stability research followed ICH guidelines. The paste was stored in a collapsible tube at various temperatures and humidity levels (25ºC ± 2ºC / 60% ± 5% RH, 30ºC ± 2ºC / 65% ± 5% RH, 40ºC ± 2ºC / 75% ± 5% RH) for three months and evaluated for appearance, pH, and Spreadability.

11. CLEANING ABILITY

Eggshells are abundant in calcium and closely resemble tooth enamel, making them ideal for testing toothpaste cleaning ability. Each toothpaste tested contained one eggshell. A beaker containing 200ml of water was brought to a boil. 15 mL of vinegar and 20 drops of red food coloring were added, respectively. A hard-boiled egg was immersed in a food coloring solution for 5 minutes, causing it to turn red. Using a permanent marker, a line was drawn along the length of the eggshell to divide it in half. A toothbrush was moistened with distilled water, and the water was brushed off before brushing one side of the egg with ten strokes. The egg was checked for any color loss. The toothbrush was cleaned with water and shaken off, then a pea-sized amount of prepared toothpaste was applied to the toothbrush and used to brush one side of the egg for 10 strokes. The egg was cleaned and tested for color removal. The method was repeated for each toothpaste that was evaluated.

12. GRITTY MATTER

A small amount of toothpaste was rubbed onto a piece of butter paper. The quantity and degree of scratches on the butter paper were categorized as absent or present.

13. ABRASIVENESS

A pea-sized amount of prepared toothpaste was applied to a clean plastic microscope slide, followed by a drop of distilled water. A clean cotton swab was rubbed back and forth on the toothpaste sample 30 times with short strokes. The slide was gently washed and dried with soft tissue. The slide was inspected using a dissecting microscope light from above. The number of scratches on the slide's surface was determined and rated on a scale of 0 (no scratches) to 5 (heavy scratches).

14. FRAGRANCE TEST

Individual observations were used to determine its approval. Five persons were polled about the appropriateness of fragrances, and their responses were recorded. The aroma of toothpaste was evaluated using the following criteria:

• The fragrance was good, comparable to the reference toothpaste.

• The fragrance was bad compared to the reference toothpaste.

IN-VITRO ANTIBACTERIAL ACTIVITY OF MURRAYA KOENIGII LEAF EXTRACTS [38-42]

Preparation of Culture Media

The nutrient media was prepared by dissolving 2.8 g of Agar-agar powder into the 100 ml of distilled water by boiling it and allow it to cool. Pour the culture media into the flask and stored it for further use.

Preparation of inoculum

The bacterial strain was prepared by inoculate the bacteria into the nutrient agar media and incubate the culture on incubator at 37ºC for 24 hours and stored for in-vitro studies.

In-vitro Antibacterial assay of Murraya koenigii leaf extracts

The antibacterial activity of the aqueous and ethanolic extracts of Murraya koenigii leaf were evaluated by the Agar Well Diffusion method.

Procedure

After the incubation period, the culture media was placed into a sterile Petri dish with a diameter of 90 mm and allowed to cool to ambient temperature before solidifying. The bacterial strain was created by spreading the inoculum onto prepared solid nutrient agar media using an inoculating loop and sterile spreader. The laboratory capillary tube was used to create an equidistance well on the nutritional agar plate that was 3mm in diameter and 5mm deep. Murraya koenigii leaf extracts, both aqueous and ethanolic, were applied to different wells of an Agar petri dish (approximately 100 µL each). It is incubated in the incubator for 24 hours at 37°C. Following the incubation period, the plates were examined for the zone of inhibition (ZOI) obtained by the plant extracts, which was measured and recorded.

RESULTS AND DISCUSSION

Phytochemical analysis of active constituents of Murraya koenigii leaves extract (aqueous and ethanol)

Tab 2: Phytochemical screening of Murraya leaves extract

S. No.	CHEMICAL TEST	AQUEOUS EXTRACT	ETHANOLIC EXTRACT
1.	Alkaloid test	+	+
2.	Carbohydrate test	+	+
3.	Glycoside test	+	+
4.	Protein test	+	-
5.	Terpenoid test	+	-
6.	Quinone test	+	-
7.	Phenol test	+	+
8.	Saponin test	+	+
9.	Flavonoid test	-	-
10.	Anthraquinone test	-	-
11.	Reducing sugar	+	+
12.	Tannins test	+	+
13.	Steroid test	-	-
14.	Coumarins test	+	+
15	Gum test	-	-

(+ = presence, − = absence)

Evaluation of formulated Murraya koenigii toothpaste

Tab 3: Evaluation parameters of formulated herbal toothpastes

S. No.	PARAMETERS	AQUEOUS EXTARCT FORMULATION	ETHANOLIC EXTRACT FORMULATION
01.	Physical examination Color Odor Smoothness	Pale green color Good Smooth	Light green color Good Smooth
02.	pH	8.26	8.30
03.	Homogeneity	Good	Good
04.	Tube inertness	Good	Good
05.	Sharp and edge abrasive particle	No solid particle	No solid particle
06.	Foamability	5 ml	5ml
07.	Moisture content	12. 3%w/v	15.6%w/v
08.	Extrudability	78 g	86 g

09.	Spreadability	5.5	5.3
10.	Stability Study	Good	Good
11.	Cleaning Ability	Good	Good
12.	Gritty Matter	Absent	Absent
13.	Abrasiveness	3	4
14.	Fragrances test	Pleasant	Pleasant

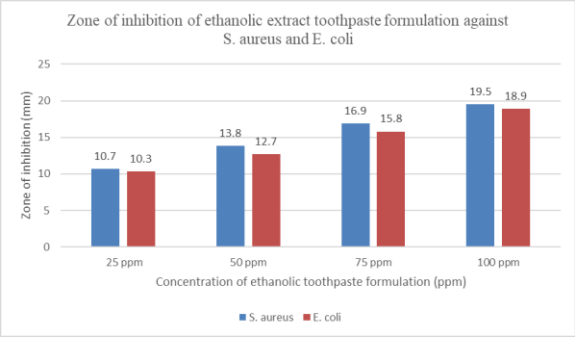
Anti-bacterial activity

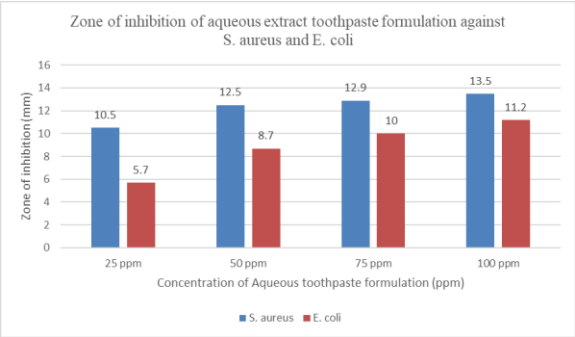
Tab 4: Antibacterial activity of aqueous and ethanolic leaf extract of formulated herbal toothpaste

S. No.	Extracts	Concentration of extracts (ppm)	Zone of (mm) Inhibition	
			Streptococcus aureus	Escherichia coli
01.	Ethanolic extract	25 ppm	10.7	10.3
		50 ppm	13.8	12.7
		75 ppm	16.9	15.8
		100 ppm	19.5	18.9
02.	Aqueous extract	25 ppm	10.5	5.7
		50 ppm	12.5	8.7
		75 ppm	12.9	10
		100 ppm	13.5	11.2

The ethanolic leaf extract of Murraya koenigii toothpaste performed better against S. aureus than the aqueous extract. At a MIC of 100 ppm, the ethanolic formulation showed an excellent ZOI of 19.5 mm, while the aqueous formulation showed a ZOI of 13.5 mm. As a result, it is possible to conclude that ethanolic extract-based dental paste possesses antibacterial properties.

The bar representation of formulated Murraya koenigii toothpaste against the gram-positive bacteria as Streptococcus aureus and gram-negative bacteria as Escherichia coli are given below.





CONCLUSION

The herbal toothpaste of Murraya koenigii leaf extract was successfully formulated by Dry gum method with various concentrations. The formulated toothpaste was evaluated for its physiochemical parameters and in-vitro antibacterial activity by Agar well diffusion method.

The research concluded that natural remedies are more acceptable and they are safer with minimum side effect than synthetic preparation. The above formulated tooth pastes totally capable to the tooth, maintain the oral hygiene and it and showed the action against pathogen i.e., anti-bacterial activity. Comparatively, the ethanolic formulated toothpaste were much better than the aqueous extract formulation. Therefore, preventing approach to the growth of microorganism inside the oral cavity. The formulated tooth paste was showing the good scope in future about dental research in natural remedies.

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