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Idendification test of phytochemical and antibacterial activity of using some medicinal plants

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Abstract
Objective: To identify phytochemical screening and Antibacterial activity against *Staphylococcus aureus* and *E. coli*, Oral bacteria individually. To prepare the agar media plates for testing of different types of bacteria.
Methods: Different types of plants are used for containing aqueous extract of Neem (*Azadirachta indica*), Clove (*Syzygium aromaticum*), Betel (*Piper betle*), Turmeric (*Curcuma longa*), Peppermint (*Mentha piperita*), and Guava (*Psidium guajava*) was used for phytochemical screening and antibacterial activity against S.aureus, B. subtilus and E. coli bacteria and identification test of inhibition zone using with different concentration of extract were used (100 mg/ml).
Results: Antibacterial activity of Neem (*Azadirachta indica*), Clove (*Syzygium aromaticum*), Betel (*Piper betle*), Turmeric (*Curcuma longa*), Peppermint (*Mentha piperita*), and Guava (*Psidium guajava*) are used as an aqueous extraction of Crude drugs against S.aureus, E.coli and Subtilus.
Conclusion: These result shows the medicinal plant extract is promising phytochemical analysis and antimicrobial effects against both gram positive and gram negative bacteria. We are used all medicinal plants are very useful for human in the present time. All plants are very good source of Antimicrobial, Antifungal, Analgesic, Antiseptic etc.

Keywords: Neem, clove, betle, turmeric etc. phytochemical studies, antibacterial activity

Introduction
India is a one of the most important country to be known for the ancient script the number system invetion 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.

Phytochemicals Found In Plants

Phytochemicals are obviously present in the plants and show biological significance while playing an important role in plants to protect themselves against various pathogenic micro-organisms by showing antimicrobial activity in plants or by blocking or killing mechanisms. Secretion varies from plant to plant (some produce more and produce some quantity in small quantities). Phytochemicals are compounds that give the plant its color, taste and smell. These compounds are considered to highly responsible for health benefits of medicinal properties and medicinal herbs. In addition, phytochemicals also contain toxic and poisonous substances found in plants.

a. Alkaloids: Alkaloids Organic is one of the largest groups of original ingredients found in plants. Those are the usually in the taste of bitter and are attributed by powerful physical action. Examples are cocaine, atropine, quinine, nicotine, morphine and caffeine.

b. Flavonoids: Flavonoids Plant is a series of ubiquitous weight in the empire, thousands of little molecular mass lipid soluble polyphenols. In vitro assays flavonoids have been shown antimicrobial, anti allergens, antithrombotic, and anti-neoplastic power.

c. Tanins: Tannin polyphenol compounds are very unstable and bitter taste. This combination has been established as a way to protect against predators from external predators by plants. Its water-soluble nature allows easy extraction and is useful in various applications in the chemical and pharmaceutical industry. Tannin-rich plants have hemostatic, antiseptic and toning properties.

d. Saponin: Saponin naturally contains glycosides of the plant. They have soaps like properties and when a mixture is mixed with water, a powder is produced. More than 100 families of plants have saponin and saponin has more than 11 classes. They can be tied with water and fats and oil. The unique chemical structure of saponin allows them to provide many possible health benefits.

e. Carbohydrate: Carbohydrates are compounds produced during photosynthesis. They have two main objectives in plants. First of all, they provide building blocks for the structural components of plants such as cellulose. Second, they are molecules that provide energy for plant growth.

f. **Phenols:** Phenolic acid, in phenolic compounds from medicinal herbs and dietary plants, Flavanoids, tannins, stilbenes, curcuminoids, coumarines, dietary plants, Flavanoids, tannins, stilbenes, curcuminoids, coumarines, lignans, quinines, and others. Various biochemistry compounds of phenol compounds responsible for their chemprovenive properties (antioxidants, anticarcinogenics, antimutagenic and anti-inflammatory effects).

g. Steroids: Plants are types of natural biological compounds found in plants steroids. Many types of plants are present in steroids and play an important role in the biological processes of plants such as resistance to resistance to environmental stress such as development and development, cell division and cold weather.

h. Proteins: Plants requisite proteins for strong improvement and development. One of the important roles of proteins in plants is to regulate phototropism and mediate mediating to highlight the reaction of plants. Proteins are also involved in energy generated reactions, intracellular structure and membrane transport.

i. Terpenoids: Terpenoids represent the largest and diverse class of chemicals among innumerable compounds produced by plants. Terpenoids use metabolites for various basic functions in plant growth and development, but use more typical chemical interactions and most of the terpenoids to protect them in the abiotic and biological environment.

Antimicrobial Activity of Medicinal Plants against Human Pathogenic Bacteria

The medicinal plant shows an expensive of antimicrobial agents. Plants are used healthful in numerous countries and are the source of many powerful and powerful medicines. A good vary of healthful plants are used to extract as raw medicine and that they have various medicinal properties, medicinal plants are considered as important sources of new chemical substances with potential therapeutic effects. Secondary metabolites of plants found sources of varied phytochemicals that would be used directly as intermediates for brand new medication production. Traditional medication should also be ready to play a greater role within the developing primary health care system of developing countries. Natural medicines are considered more acceptable to the human body than modern synthetic medicines. In order to provide adequate health care services to the rural people, getting the maximum benefit from the traditional system of medicine is the most important factor. Nature has long been an important source of pharmacological agents. Based on the use of conventional pharmaceutical pharmacological in plants, effective numbers of modern drugs have been separated or derived from different sources. The considering potential of the plants form of the antimicrobial drugs as a source of the antibacterial agents of medicinal plants. Oral microbiology is the study of the microorganisms of the oral cavity and their interactions between oral microorganisms or with the host. The environment present in the human mouth allows the growth of characteristic microorganisms found there. It provides a source of water and nutrients, as well as a moderate temperature. Resident microbes of the mouth adhere to the teeth and gums to resist mechanical flushing from the mouth to stomach where acid-sensitive microbes are destroyed by hydrochloric acid.

Materials and Method

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 ^{0}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.

Principle

After once crushing the crushed sample was mixed in distilled water with the assistance of magnetic stirrer and therefore the mixture was any filtered with the assistance of muslin cloth.

Procedure

- Fine sample was weighed and mixed properly in distilled water.
- Mixing was done magnetic stirrer for 10 minutes.
- The mixed solution was filtered with help of muslin cloth.
- The filtrate was taken
- The prepared extract was stored in conical tubes at 4^0C temp for further use.

A. Qualitative tests

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 road
- 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 $CHCl_3$ 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-green in experienced or red coloration indicated the presence of quinones.

Isolation of oral bacteria

- 15 ml nutrient agar was prepared
- Take a cotton swab that has already put in UV light
- Moisten a sterile cotton swab in sterile water and dip it into a fresh saliva
- Remove the excess water by pushing the swab against the test tube wall
- dip it into a fresh saliva
- Spread swab on nutrient agar plate
- Incubate the inoculating agar for 24-48 hrs. at 37 in an inverted position
- Observe the morphology of bacterial colony

Identification on Isolated Microorganism

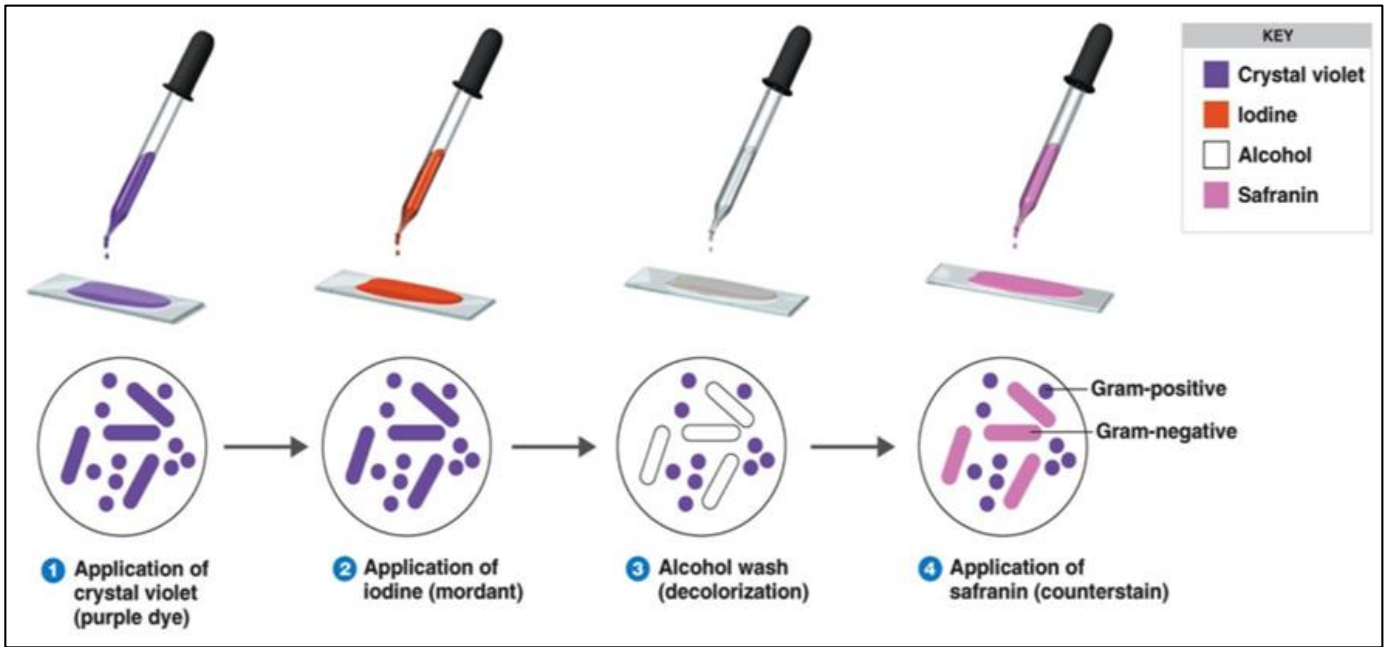
Gram Staining:

Principe: Once the microorganism is stained with primary stain antibacterial drug and glued by the mordant, a number of the microorganisms are ready to retain the first stain and a few are decolorized by alcohol. The cell walls of gram positive microorganism have a thick layer of protein-sugar complexes known as peptidoglycan and super molecule content is low. Decolorizing the cell causes this thick cell membrane to dehydrate and shrink, that closes the pores within the cell membrane and prevents the stain from exiting the cell. That the ethyl alcohol cannot take away the Crystal Violet-Iodine complex advanced that's absolute to the thick layer of peptidoglycan of gram positive microorganism and seems blue or purple in colour.

In case of gram negative microorganism, cell membrane conjointly takes up the CV-Iodine advanced however thanks to the skinny layer of peptidoglycan and thick outer layer that is made of lipids; CV-Iodine advanced gets washed off. Once they are exposed to alcohol, decolorize dissolves the lipids within the cell membrane, that permits the crystal violet-iodine advanced to leach out of the cells. Then once more stained with safranin, they take the stain and seem red in color.

Procedure

- Exploitation sterile technique, a drop of saline was placed on the slide.
- A little quantity of a microorganism colony was then transferred to the drop of saline with a sterile cooled vaccinating loop.
- A smear was then ready by combining and spreading the microorganism by suggests that of a circular motion of the loop.
- The smear was then allowed to air dry followed by heat fixation.
- Gently flood smear with antibacterial drug and let indicate for 1 minute.
- Tilt the slide slightly and gently rinse with tap water or employing water a wash bottle.
- Discolourise exploitation 95% ethyl alcohol or dissolving agent. Tilt the slide slightly and apply the alcohol call in drop for 5 to 10 seconds till the alcohol runs nearly clear. Take care not to over-decolorize.
- Immediately rinse with water.
- Gently flood with safranin to counter-stain and let indicate for 45 seconds.
- Tilt the slide slightly and gently rinse with tap water or water employing a wash bottle.
- Blot dries the slide with inebriated paper.
- Read the smear employing a light-microscope underneath oil-immersion.



Gram Staining Procedure

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 all the reagent of NAM and dissolved in 60 ml water.
- Heated with agitation to dissolve the constituents properly.
- Autoclaved at 121 ºC 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.

Result & Discussion

Phytochemical Test

Table 1: Phytochemical Test

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

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

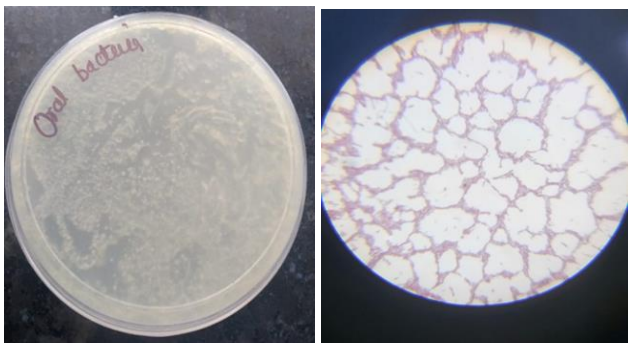
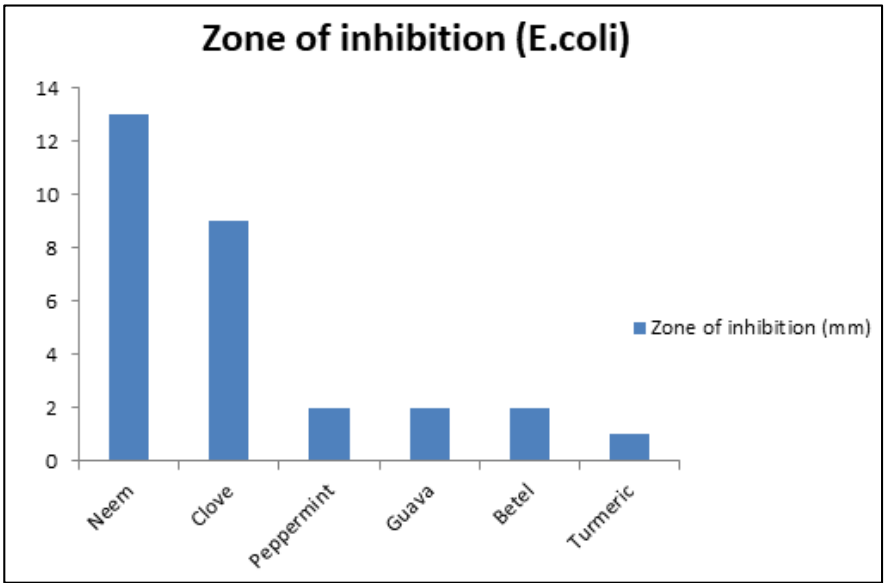


Fig 1: Oral Bacteria plate and gram stain result

Table 2: Zone of inhibition of the given plant samples against *E. coli*

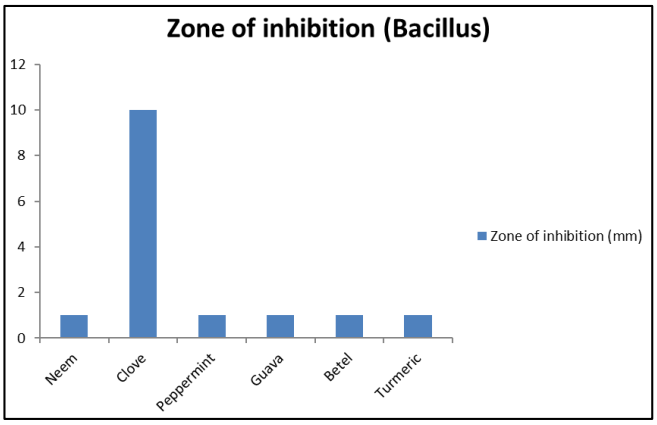
1	Sample	Zone of inhibition (mm)
2	Neem	13
3	Clove	9
4	Peppermint	2
5	Guava	2
6	Betel	2
7	Turmeric	1



Graph 1: Depicting Antibacterial activity of plant extract of Neem (*Azadirachta indica*), Peppermint (*Mentha piperita*), Turmeric (*Curcuma longa*), Clove (*Syzygium aromaticum*), Betel (*Piper betle*) and Guava (*Psidium guajava*)

Table 3: Zone of inhibition of the given plant samples against Bacillus subtilus

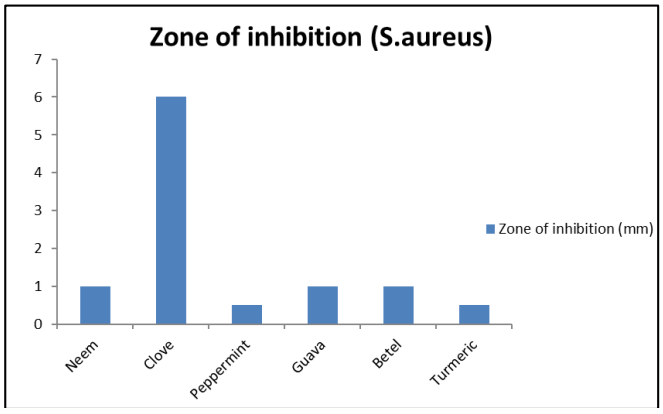
1	Sample	Zone of inhibition (mm)
2	Neem	1
3	Clove	10
4	Peppermint	1
5	Guava	1
6	Betel	1
7	Turmeric	1



Graph 2: Depicting Antibacterial activity of plant extract of Neem (*Azadirachta indica*), Peppermint (*Mentha piperita*), Turmeric (*Curcuma longa*), Clove (*Syzygium aromaticum*), Betel (*Piper betle*) and Guava (*Psidium*

Table 4: Zone of inhibition of the given plant samples against *S.aureus*

S. No.	Sample	Zone of inhibition (mm)
1	Neem	1
2	Clove	6
3	Peppermint	0.5
4	Guava	1
5	Betel	1
6	Turmeric	0.5



Graph 3: Depicting Antibacterial activity of plant extract of Neem (*Azadirachta indica*), Peppermint (*Mentha piperita*), Turmeric (*Curcuma longa*), Clove (*Syzygium aromaticum*), Betel (*Piper betle*) and Guava (*Psidium*

Discussion

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 and as a analgesic, for pain management throughout dental work, and for a complication of the 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.

Conclusion

This extraction was tested for antibacterial drug against Staphylococcus aureus, Escherichia coli and oral micro flora micro the efficiency was qualitatively and quantitatively assessed by the presence or absence of a zone of inhibition and zone diameter values. The developed sample extract was inhibited extremely vital result towards the entire tested microorganism, whereas the negative controlling doesn't turn out visible 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. 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 sample produced active constituents of the extract and therefore activity also well maintained once it absolutely. This was sensible sign to try to additional studies thereon to create it together of the flavoring agent and medicinal activity for the treatment of oral microorganism infections.

References

1. 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.
2. 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.
3. Collins L *et al*., Instant tooth whitening from a silica toothpaste containing blue covarine. J of Dentistry. 2006; 36(8):21-5.
4. Mamatha D.IOSR Journal of pharmacy and biological Sciences (IOSR- JPBS) 2017; 12(6):V
5. 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 2010; 4(12):1182-1186.
6. Kandarp Dave. International Journal of chemistry and Pharmaceutical Sciences, 2014; 2(3):666-673.
7. 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.
8. Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anti-cancer agents. Fitoterapia. 2010; 81(7):703-14. Doi 10.1016/j.fitote.2010.06.004. Epub 2010 Jun.

9. Mahendra sekar *et al* International journal of pharmaceutical and clinical research. 2016; (8):1155-1158.
10. Meyers AI *et al*., The surface effect of dentifrices .Australian Dent J. 2000; 45(2):118-124.
11. Mridul Haque Journal of chemical and Pharmaceutical Research. 2014; 6(3):1279-1285.
12. Nitin CM *et al*., Chitosan-based toothpaste: As novel oral hygiene product. Indian J Dent Research. 2010; 21(3).
13. Okpalugo J *et al*., Toothpaste formulation efficacy in reducing oral flora. Trop. J of Pharm. Research. 2009; 8(1):71-7.
14. 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.
15. Praphasri R *et al*., Effects of fluoride dentifrice on remineralization of demineralized primary enamel. South East Asian. J Trop. Med. Public Health. 2010; 41(1).
16. Ramya Kuber. international journal of pharmaceutical and clinical research, 2015.
17. 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
18. Siddharth Sharma. International journal of pharmaceutical Research & Allied Sciences. 2014; 3(2):30-39.
19. 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. 2010; 2(2)
20. 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.
21. Mangilal T. International journal of Ayurvedic & herbal medicine. 2016; 6(3):2266-2273.
22. Vaibhav Shende. European journal of pharmaceutical and medical research. 2017.
23. Wei Hu, Yan HZ, Wang Q, William D, Margaret P. Effects of fluoride toothpaste on etched enamel of orthodontic patients. Chin. J of Dent. Research. 1999; 2(3):79-83.
24. http://article.sciencepublishinggroup.com/pdf/10.11648.j.sjph.20170506.11df
25. https://www.msn.com/en-in/health/medical/arthritis-causes-symptoms-and-treatments/ss-AApOxZE
26. http://www.indiaparenting.com/childs-healthcare/35_1237/cough-and-cold.html
27. https://link.springer.com/article/10.1007%2Fs13197-013-0953-7
28. http://agropedia.iitk.ac.in/content/store-turmeric-and-sell-april-may
29. http://www.crri.nic.in/News/crri_news_rpast.htm
30. http://www.tandfonline.com/doi/full/10.1080/03235408.2010.526775?scroll=top&needAccess=true
31. http://www.scholarsresearchlibrary.com/articles/role-of-plant-growth-promoting-rhizobacteria-for-biocontrol-ofphytopathogens.pdf
32. http://www.indianspices.com/spice-catalog/turmeric-1
33. https://www.researchgate.net/publication/301644533_Curcuma_longa_Linn_A_Phytochemical_and_Phytopharmacological_Review
34. https://www.scribd.com/document/60328030/Write-up
35. http://varcophyto.com/rnd_ingredient
36. http://ndpublisher.in/admin/issues/IJAEBV7N3o.pdf
37. http://www.mardaturmeric.com/why-turmeric.php
38. http://www.interactive-biology.com/3982/classification-of-secondary-metabolites-how-plants-and-humans-use-them-2/
39. http://article.sciencepublishinggroup.com/html/10.11648.j.jps.s.2015030101.18.html
40. https://www.scribd.com/presentation/48160021/Part-I
41. http://tycomputer.com.ng/phytochemicals-2/
42. https://link.springer.com/article/10.1007/s00374-012-0691-4
43. https://www.herbal-supplement-resource.com/phytochemicals-herbs.html