



## Studies on Antimicrobial Activity and Phytochemical Analysis of *Eugenia Caryophyllata* Essential Oil Against Dental Pathogens *Streptococcus Mutans* and *Candida Albicans*

S.P. Mohana Priya<sup>1</sup>, P. Vadivukkarasi<sup>2</sup>

<sup>1</sup>Research Scholar, PG and Research Department, Department of Microbiology, Kamban college of Arts and Science for Women - Tiruvannamalai.

<sup>2</sup> Assistant professor, PG and Research Department, Department of Microbiology, Kamban college of Arts and Science for Women - Tiruvannamalai.

### ABSTRACT

*Eugenia caryophyllata* (Cloves) are famous for its spices and widely used in cooking throughout the world especially in the Europe and Asia. Besides, cloves are also essential in the manufacturing of herbal medicine such as Indian, Ayurvedic medicine and Chinese medicine. The health benefits of *Eugenia caryophyllata* essential oil can be attributed to its antimicrobial, antifungal, antiseptic, antiviral and stimulating properties. The clove oil is used for treating a variety of health disorders including toothaches. Dental carries is an oral disease of bacterial origin that causes demineralization and destruction of tooth structure. *Streptococcus mutans* is a Gram positive bacteria that play a vital role in the formation of tooth decay. This commensal bacteria of the oral cavity is found in saliva, buccal mucosa, tongue, gingival sulcus. A diploid Gram positive fungus known as *Candida albicans* is the most common one that found in the oral cavity both in commensal form and in case of oral Candidiasis. With the aid of Phytochemical analysis, Disc diffusion method, Minimum Inhibitory Concentration and Minimum Bactericidal Concentration methods the present study was created as to certain the function of the *Eugenia caryophyllata* essential oil, in the in vitro antimicrobial activity against *Streptococcus mutans* and *Candida albicans*. The antimicrobial activity and Minimum Inhibitory Concentration of the samples were assessed using Muller Hinton Agar plates with direct oil and solvent extract such as methanol. After 24 hours of incubation on the plates at 37°C, the zone of inhibition was measured and tabulated.

**Keywords:** *Eugenia caryophyllata*, Antimicrobial activity, *Streptococcus mutans*, *Candida albicans*

### INTRODUCTION

The fragrant dried flower buds known as *Eugenia caryophyllata* (Cloves) come from an evergreen tree in the *Myrtaceae* family that is indigenous to the Maluku Islands in East Indonesia. It has been used as a traditional spice in Asia and Europe for countless years. They are the unopened inflorescences that are between 1/2 - 3/4 inches long and contain between 14-20% essential oil.

Dentists have employed *Eugenia caryophyllata* as an analgesic in the treatment of painful and infectious illness of the oral cavity as well as a dressing for small dental lesions. Additionally, it has been utilized in medical and dental procedures as an antispasmodic and general antiseptic. The purpose of the current study was to evaluate the antimicrobial capabilities of cloves oil, because early childhood carries are related with *Candida albicans* and dental carries in adults are connected with *Streptococcus mutans*.

The most significant and typical application of clove oil is in tooth hygiene. The essential oil of *Eugenia caryophyllata* is employed in dentistry as an anodyne for dental emergency. It is a key component in a number of toothpaste, mouthwash, and oral care products. It is used as a pain reliever in dentistry. It is used on the gums to ease tooth pain. In addition, it has a significant amount of eugenol, which is used to lessen the pain in the nerves during extractions, typically when there is a problem called a "dry socket". When Eugenol and Zinc oxide are combined, cement is created, which can then be placed to the tooth to temporarily fill cavities.

*Streptococcus mutans*, the name of the commensal bacterium of the oral cavity, is present in saliva, buccal mucosa, tongue, and the gingival sulcus. They are most frequently discovered in dental plaque that has adhered to the surface of the tooth. The presence of additional oral species that are capable of producing carries is indicated by the fact that 10% of the patients with carries do not have large levels of *Streptococcus mutans*. The same goes for *Streptococcus mutans*, which has a strong correlation with tooth decay lesions and may produce more acidic isolates from decay lesions than from healthy teeth.

The word "candid," which means "white" in Latin, is where the word "candid" comes from. The commensal, non-pathogenic *Candida* spores transform into invasive, pathogenic pseudohyphae when there is a disruption in the flora's delicate equilibrium or when the host becomes weak. There are various

species of *Candida*, but *Candida albicans* is the most common one that is found in the oral cavity, both in commensal form and in cases of oral candidiasis. This species is thought to make up more than 80% of all yeast isolates from oral bacteria.

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## MATERIALS AND METHODS

The present investigation was under taken Studies on Antimicrobial activity and Phytochemical analysis of *Eugenia caryophyllata* essential oil against clinically important dental pathogens *Streptococcus mutans* and *Candida albicans*. The study was carried out in Kamban college of arts and Science For women, Thiruvannamalai.

### CLEANING OF GLASSWARE

All the glass wares were immersed in a cleaning solution for a few hours. Then the glassware's were washed thoroughly with tap water, followed by detergent solution and finally rinsed with distilled water. The cleaned glassware was dried in a hot air oven, to ensure that it is free from contaminants and stored.

### STERILISATION OF MATERIALS

Glass wares and culture media were sterilised at 121°C for 15 minutes at 15 lbs.

### COLLECTION OF TOOTH SAMPLE

The samples was collected from tooth infected patients in Arunai Medical College and Hospital in Thiruvannamalai. The specimen should be collected under aseptic conditions. Label the specimen appropriately, all clinical specimens should be considered as potential biohazards and should be handled with care using universal precautions. Finally, observe the microbial growth.

#### 1. ISOLATION AND IDENTIFICATION OF BACTERIA FROM TOOTH INFECTED PATIENTS

The bacteria present in Tooth samples of tooth infected women patients were cultured in the laboratory media. The isolated bacterial culture was identified by Gram staining technique and Biochemical tests.

#### 2. ISOLATION AND IDENTIFICATION OF YEAST FROM TOOTH INFECTED SAMPLE:

The yeast present in tooth samples of tooth infected patients were cultured in laboratory media. The isolated yeast culture was identified by Gram Staining, Germ tube test, Chlamydospore formation and Sugar assimilation test.

### COLLECTION OF CLOVE OIL

Clove oil was collected from Siddha medical shop, Thiruvannamalai, Tamilnadu, India.

#### 3. PHYTOCHEMICAL TEST:

The clove oil was tested in order to find out presence of active phytochemical compounds by use of aqueous and methanol extracts.

#### 4. DISC DIFFUSION METHOD

1. The disc diffusion method is widely used to evaluate the antimicrobial activity of essential clove oil.
2. The Muller Hinton agar medium was prepared and poured into sterile petriplate and get solidified. The organisms *Streptococcus mutans* and *Candida albicans* culture was spread into agar surface with help of sterile cotton swab.
3. A sterile filter disc, diameter 6 mm (Whatman paper No. 3) was placed in the plate. Three microliters of the essential oil were dropped on each paper disc.
4. A standard disc of erythromycin was used as positive control. An unfilled paper disc was used as negative control.
5. The plates were incubated at 37°C for 24 hours.
6. The inhibitory effect of the essential oil against each test strain was determined by measuring the diameter zones (in millimetres) around the discs. Each experiment was carried out in triplicate.
7. Results were tabulated.

#### 5. MINIMUM INHIBITORY CONCENTRATION (MIC)

1. The turbidity method or tube dilution method was used for determination of minimum inhibitory concentration.
2. The extracts were taken at different concentrations of 5, 10, 15, 20 and 25 µl/ml in test tubes containing 1 ml sterile Muller Hinton broth.
3. Then, 0.1 ml of bacterial suspension were inoculated in test tubes.
4. The control tube was maintained.

5. All the tubes were then incubated at 37°C for 24 hours and then examined for growth by observing turbidity.

## 6. MINIMUM BACTERICIDAL CONCENTRATION (MBC)

1. A loopful of broth was collected from the each tube of Minimum Inhibitory Concentration (MIC), that are used for determination of Minimum Bactericidal Concentration (MBC).
2. The broth was inoculated into a sterile nutrient agar by streaking.
3. The inoculated plates were incubated at 37°C for 24 hours.
4. After incubation the concentration at which there was no visible growth, was noted as the minimum bacterial concentration.

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## RESULT AND DISCUSSION

The present study reveals that the tooth sample were collected from tooth infected patients, at Arunai Medical College and Hospital, Thiruvannamalai. The sample inoculated into nutrient broth, peptone water and Sabouraud Dextrose broth. After incubation observe the microbial growth.

Based on their morphological shape, visualized by gram staining technique, the Gram positive cocci was observed. The nutrient broth culture was inoculated into differential selective media and observed the colony morphology. The results shown in **Table: 1**.

The bacterial culture was inoculated into a selective media of Mitis Salivarius Agar. After incubation, the *Streptococcus mutans* form pale blue, raised, convex, undulate and opaque colonies. In this study, the isolated culture was further characterized by biochemical test, Indole Test, Methyl Red Test, Voges Proskauer Test, Citrate utilization Test, Urease Test, Triple Sugar Iron Test, Catalase Test, Oxidase Test. The results shown in **Table: 2**.

The ability of microorganism *Streptococcus mutans* ferment Fructose, Glucose, Lactose, Maltose and Sucrose and could not ferment Ribose and Xylose, the results shown in **Table: 3**.

On Sabouraud Dextrose Agar, the plate shows *Candida albicans* a large, round, white or cream colour colonies. In gram stained smears the *Candida albicans* appears Gram positive budding yeast cells.

It is further characterized by Germ tube Test. It shows the Germ tube, called pseudo-germ tube, where they adhere to the mother cells. The Germ tube test is routinely used for the rapid identification of *Candida albicans*. **Fig:1**

The pathogens of *Candida albicans* has the special ability to form thick-walled cells, termed Chlamydo spores, which is produced under very specific invitro condition.

It is useful test to discriminate between *Candida albicans* and *Candida dubliniensis* (**Mosca C O et al., 2003**).

The *Candida albicans* ferment Arabinose, Glucose, Sucrose not Lactose and Maltose. The results shown in **Table: 4**.

The phytochemical analysis of *Eugenia caryophyllata* (clove oil) revealed the presence of Phenols, Tannins and Terpenoids while Alkaloids, Cardiac glycosides, Flavonoids, Resins and Anthraquinones is absent.

The methanol extracts of clove oil revealed the presence of Alkaloids, Flavonoids, Phenols, Tannins and Triterpenes while Cardiac glycosides, Resins and Anthraquinones is absent, the results shown in **Table: 5**.

The analysis of the preliminary phytochemical test of the oil showed the presence of chemical constituents like Alkaloids, Flavonoids, Phenols, Tannins, Terpenoids. Alkaloids are pharmacologically active as they have physiological effects on human as well as other animals and serves as therapeutic drugs. (**Kamalpreet kaur et al., 2019**).

According to the findings of this study's investigation, clove oil extract included phytochemicals with established pharmacological actions. This study showed that clove oil extracts has large number of phytochemicals that are importance for dental health. (**Parekh J et al., 2005**).

The oil of *Eugenia caryophyllata* extracts for evaluation of antimicrobial activity against oral pathogens. Results showed that direct oil there was a moderate activity against *Streptococcus mutans* (12 mm) and *Candida albicans* (20 mm).

Disc diffusion analysis of antimicrobial activity of direct clove oil that showed maximum zone in *Candida albicans* with 20 mm zone and less in *Streptococcus mutans* with 14 mm zone (**Kamal Rai Aneja et al., 2010**)

The methanol extract had strong activity against *Streptococcus mutans* (18 mm) and *Candida albicans* (24 mm) respectively. Based on this results methanolic extract more sensitive than direct oil. The results shown in **Table: 6 and Fig: 2**

The results obtained from our study shows that the both extracts have a very good antimicrobial activity against dental pathogen of *Streptococcus mutans* and *Candida albicans*. (**Katarzyha Rajkowska et al., 2017**)

Results of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration are shown in **Table: 7**. The Minimum Inhibitory Concentration and Minimum Bactericidal concentration of direct oil of *Streptococcus mutans* are 25 µl/ml and 20 µl/ml, while the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration at direct oil of *Candida albicans* are 20 µl/ml and 15 µl/ml respectively.

The MIC values of the extracts which showed antimicrobial activity. All activities of *Eugenia caryophyllata* extracts were recorded against the isolates, and the MIC values were determined as the lowest concentration of the direct clove oil and methanolic extract of clove oil that showed inhibition against the *Streptococcus mutans* and *Candida albicans* (**Radhika Joshi et al., 2010**)

The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of methanolic extract of *Candida albicans* are 10 µl/ml and 5 µl/ml, while the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of methanolic extract of *Candida albicans* are both 5 µl/ml respectively.

The lower Minimum Inhibitory Concentration and Minimum Bactericidal Concentration value of methanol extract proves its better efficiency against the tested pathogens of *Candida albicans* (5 µl/ml) while methanol extract was able to inhibit the growth of *Streptococcus mutans* (10 µl/ml) at higher concentration.

The lowest methanol concentration of the essential oil that completely inhibited the growth of *Candida albicans* (**Gopinath prakasam et al., 2014**).

The MBC values were determined as the Minimum Bactericidal Concentration of the direct clove oil and methanolic extract of the clove oil that showed inhibition against the *Streptococcus mutans* and *Candida albicans* (**Radhika Joshi et al., 2010**)

**TABLE 1: OBSERVATION OF COLONIES FROM SELECTIVE MEDIA**

S.NO	SELECTIVE MEDIA	COLONY MORPHOLOGY
1	Mitis Salivarius Agar	Pale blue colonies
2	Sabouraud Dextrose Agar	White or Cream colonies

**TABLE 2: BIO CHEMICAL TEST RESULTS**

S.NO	NAME OF THE TEST	<i>Streptococcus mutans</i>
1	Gram stain	Gram Positive cocci, in chains
2	Indole test	-
3	Methyl red test	-
4	Voges Proskauer test	+
5	Citrate utilization test	-
	Urease test	-
7	Triple sugar iron test	+
8	Catalase test	-
9	Oxidase test	+

(+) Present

(-) Absent

**TABLE 3: SUGAR FERMENTATION TEST RESULTS OF *Streptococcus mutans***

S.NO	SUGARS	<i>Streptococcus mutans</i>
1	Fructose	+
2	Glucose	+
3	Lactose	+
4	Maltose	+
5	Sucrose	+
6	Ribose	-
7	Xylose	-

**TABLE 4: SUGAR ASSIMILATION TEST RESULTS OF *Candida albicans***

S.NO	SUGARS	<i>Candida albicans</i>
1	Arabinose	+
2	Glucose	+
3	Lactose	-
4	Maltose	-
4	Sucrose	+

**TABLE 5: PHYTOCHEMICAL ANALYSIS TEST RESULTS**

S.NO	PHYTOCHEMICAL COMPONENTS	DIRECT OIL	METHANOL EXTRACT
1	Alkaloid (Mayer's Test)	-	+
2	Cardiac glycosides (keller killiani Test)	-	-
3	Flavonoids (Ammonium Test)	-	+
4	Phenols	+	+
5	Quinone	-	-
6	Resins	-	-
7	Tannins (Ferric chloride Test)	+	+
8	Terpenoids	+	+

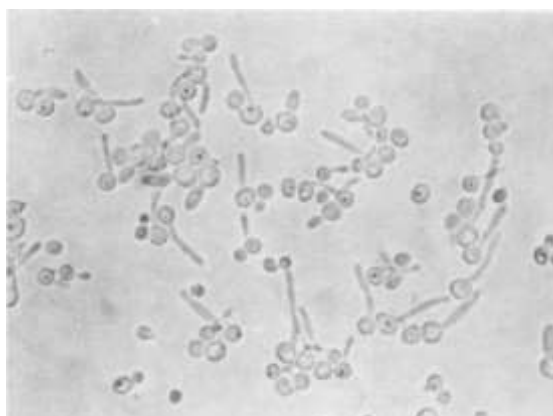
(+) Present            (-) Absent

**TABLE 6: DISC DIFFUSION METHOD RESULTS**

S.NO	ORGANISMS	ZONE OF INHIBITION IN mm	
		DIRECT OIL	METHANOL EXTRACT
1	<i>Streptococcus mutans</i>	12 mm	18 mm
2	<i>Candida albicans</i>	20 mm	24 mm

**TABLE 7: RESULTS OF Minimum Inhibitory Concentration (mic) and Minimum Bactericidal Concentration (mbc)**

S.NO	ORGANISMS	CONCENTRATION OF EXTRACT ( $\mu$ l/ml)			
		DIRECT OIL		METHANOL EXTRACT	
		MIC	MBC	MIC	MBC
1	<i>Streptococcus mutans</i>	25	20	10	5
2	<i>Candida albicans</i>	20	15	5	5

**Fig 1: Germ tube Test of *Candida albicans***

**Fig 2: Antimicrobial activity of Methanol extract against *Candida albicans***


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## CONCLUSION

The results obtained from this study showed that clove essential oil can be considered good source of natural compound for antibacterial and antifungal activities. It is a powerful and easily available source with low toxicity and high efficacy for therapeutic uses. Therefore, it can be concluded that clove essential oil was found to be effective against oral pathogenic organisms like *Streptococcus mutans* and *Candida albicans*. The methanolic extract of clove oil was more effective than direct clove oil. The antimicrobial activity of clove oil extract at different concentration was screened by disc diffusion technique, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). So, from this study it can be concluded that clove oil possess antimicrobial activity. It can be used as antimicrobial supplement in the developing countries towards the development of therapeutic agents. Clove has pharmacological effects that include antimicrobial, antidiabetic, antioxidant, anti-inflammatory, analgesic, anti-cancer and anesthetic properties. These biological processes can be highly beneficial for illness prevention and therapy.

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## SUMMARY

The current study was antimicrobial activity and phytochemical analysis of *Eugenia caryophyllata* essential oil against clinically important dental pathogens *Streptococcus mutans* and *Candida albicans*. It was identified by collection of tooth sample from tooth infected patients. The sample inoculated into Nutrient broth for bacteria and Sabourand dextrose Agar for fungi. After incubation observe the microbial growth. Further analysis by Gram staining, Biochemical test and Germ tube test. Further carried out by Phytochemical analysis, Disc Diffusion Method, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). *Eugenia caryophyllata* essential oil inhibit the growth of *Streptococcus mutans* and *Candida albicans*. So, the essential oil is used to medicinal purposes. The Clove oil has historically been used for several therapeutic agents. The occurrence of dental caries mainly associated with oral pathogens especially cariogenic bacteria. The clove oil has Alkaloids, Cardiac glycosides, Flavonoids, Phenols, Tannins, Triterpenes which may benefit oral health by reducing the risk of tooth infections.

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