PHYTOCHEMICAL AND ANTIMICROBIAL POTENTIAL IN LEAVE EXTRACTS OF *EUCALYPTUS CITRIODORA* & *LAURUS NOBILIS* - FORMULATION OF HERBAL TOOTHPASTE AGAINST ORAL PATHOGENIC BACTERIA

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Abstract

Dental caries and periodontal diseases are two of world's most significant oral health issues. Some microorganism flourish in the mouth despite presence of saliva, mechanical forces of chewing and eating. These microorganism can harm teethes with infections which can extend beyond mouth and harm throughout body. To enhance the oral health and hygiene toothpaste, mouthwash are regularly used, herbal toothpaste have various antimicrobial activity which helps to manage cure oral health problem, illness. The present study focused to analyze the phytochemical and antibacterial activity of *Eucalyptus citriodora* and *Laurus nobilis* against oral infection causative bacteria's *S. aureus, E. coli* & *K. pneumonia*. Phytochemical, antioxidant & antibacterial properties has found in both the plants. The finding imply that the use of *Eucalyptus citriodora* & *Laurus nobilis* may be employed as a antibacterial agent and formulation of herbal toothpaste.

Key words: Periodontal diseases, antibacterial, herbal toothpaste.

INTRODUCTION

The increasing prevalence of oral diseases and the emergence of antibiotic-resistant bacteria have necessitated the exploration of novel antimicrobial agents. In this context, the potential of phytochemicals derived from plant extracts as alternative therapeutic agents has garnered significant attention. *Eucalyptus citriodora* and *Laurus nobilis*, known for their aromatic properties and traditional medicinal uses, have been reported to possess a wide range of biological activities, including antimicrobial effects (1). This research aims to investigate the

phytochemical composition and antimicrobial potential of leave extracts from *Eucalyptus citriodora* and *Laurus nobilis*, focusing on their efficacy against oral pathogenic bacteria. The ultimate goal is to formulate a herbal toothpaste that leverages these plant extracts' natural antimicrobial properties to offer a safer, effective alternative to conventional oral care products, which often contain synthetic antimicrobial agents that can contribute to antibiotic resistance and have adverse environmental impacts. By harnessing the innate power of these plant extracts, this study endeavors to contribute to the field of natural product research and the development of innovative, eco-friendly oral healthcare solutions (2). Microbial diseases of the mouth and oral cavity are primarily caused by bacteria, viruses, fungi, and in some cases, protozoa. These microorganisms can affect various parts of the oral cavity, including the teeth, gums, palate, tongue, and the mucous membranes lining the mouth (3). The following are some of the most common microbial diseases affecting the oral region includes dental Caries (Tooth Decay): Caused by bacteria such as Streptococcus mutans and Lactobacillus, which ferment dietary sugars to produce acid that demineralizes the tooth enamel (4,5,6). Periodontal Disease (Gum Disease):Infections of the structures around the teeth, including the gums, periodontal ligament, and alveolar bone. Primarily caused by bacteria like Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans (7,8,9). Gingivitis: A mild form of periodontal disease, causing inflammation and bleeding of the gums, often resulting from the accumulation of plaque. Trench Mouth (Necrotizing Ulcerative Gingivitis): A severe gum infection causing painful ulcers, bleeding, and bad breath. It is associated with poor oral hygiene, stress, and malnutrition (10). The mouth's warm, moist environment is conducive to the growth of microorganisms, making oral hygiene critically important for preventing microbial diseases. Regular dental check-ups, proper brushing, flossing, and avoiding tobacco and excessive sugar can help maintain oral health and prevent these conditions. Moreover, understanding the microbial etiology of oral diseases is crucial for developing effective treatments and preventive strategies (11,12,13).

Material & Method:

Plant material and collection:

The plant leafs of *Eucalyptus citriodora* collected from Prayagraj (District Uttar Pradesh) during October 2023. *Laurus nobilis* leafs obtained from general store shop. Leaves were cleaned in

sterile distilled water & dried at room temperature in air. *Eucalyptus citriodora* and *Laurus nobilis* leaves were crushed into fine powder using mortar & pestle.

Preparation of leave extracts:

All dried plant material weighing 10gm, extracted in 100ml distilled water for 6 hours on low heat, then filtered through muslin cloth as well as centrifuged for 15 minutes at 6400rpm. Supernatants were collected in flasks as well as autoclaved at 121°C for 15 lbs of pressure, after which samples were kept at 4°C until needed.

Phytochemical analysis: Qualitative and Quantitative analysis of phytochemical done with various Biochemical tests which includes of saponin, tannin, Protein, Phenolics, Flavonoids with extracts of Eucalyptus citriodora and Laurus nobilis leaves. [14,15,16]

Bacterial Culture: An oral swab sample taken from teeth of person who experiencing cavity problems. samples taken in morning, before cleaning teeth. The bacteria E. Coli & Klebseilla pneumonia were cultivated in lab on EMB medium at 37 °C for 24-48 hours. Biochemical tests has performed for the identification of the bacteria's.

Antibiotic susceptibility testing: Antimicrobial susceptibility performed as normal practise in all microbiology labs as result of development of numerous antimicrobials for treating range of illnesses. Disc diffusion method were used for the antibiotic susceptibility testing. (17)

Formulation of Herbal Toothpaste: Using home mixer, all herbal materials were dried as well as pulverised. Ingredients were weighed as well as placed in mortar in desired amount. Water , combined with calcium carbonate, sodium lauryl sulphate, as well as glycerin. aforesaid combination , supplemented with acacia. Drop by drop, this solution , put to mortar containing herbal components as well as thoroughly triturated until paste consistency , achieved.(18)

Result:

Phytochemical test results: Qualitative as well as quantitative test has been performed as well as following results have observed as per table No 1. Quantitative estimation: Phenolic concentration, estimated in *Eucalyptus citriodora* & *Laurus nobilis* from standard graph of Gallic acid with different concertation shown in Fig 1. concentration , observed 0.07 mg/ml as

well as 0.017 mg/ml respectively Tannin concentration, estimated in *Eucalyptus citriodora* & *Laurus nobilis* from standard graph of Tannic acid with different concertation shown in Fig 1. concentration , observed 59.19 µg/ml as well as 24.65 µg/ml respectively. Flavonoid typical graph of quercetin with varying concertation, used to estimate concentration in *Eucalyptus citriodora* & *Laurus nobilis* concentrations were found to be 131.88g/ml as well as 83.76 g/ml.

Table 1: RESULTS OF QUALITATIVE ANALYSIS TEST:

S No.	Qualitative test	*Eucalyptus globous*	*Laurus nobilis*
1	Saponin	-	+
2	Tannin	+++	+
3	Flavonoid	+++	+
4	Keller kilani	+++	++
5	Ninhydrin test	-	-
6	Anthraquinone	-	-
7	Naphthoquinone	+	-
8	Diterpenes	-	+
9	Wagner's Test	-	+
10	Alcl3(flavonoid)	++	+
11	Phlobatannin	-	-
12	Molisch test	+	+
13	Benedict's test	+	++
14	Lieberman Test	+	-

15	Quinone's test	+	-
16	Xanthoprotein test	-	+
17	Vit C	-	+

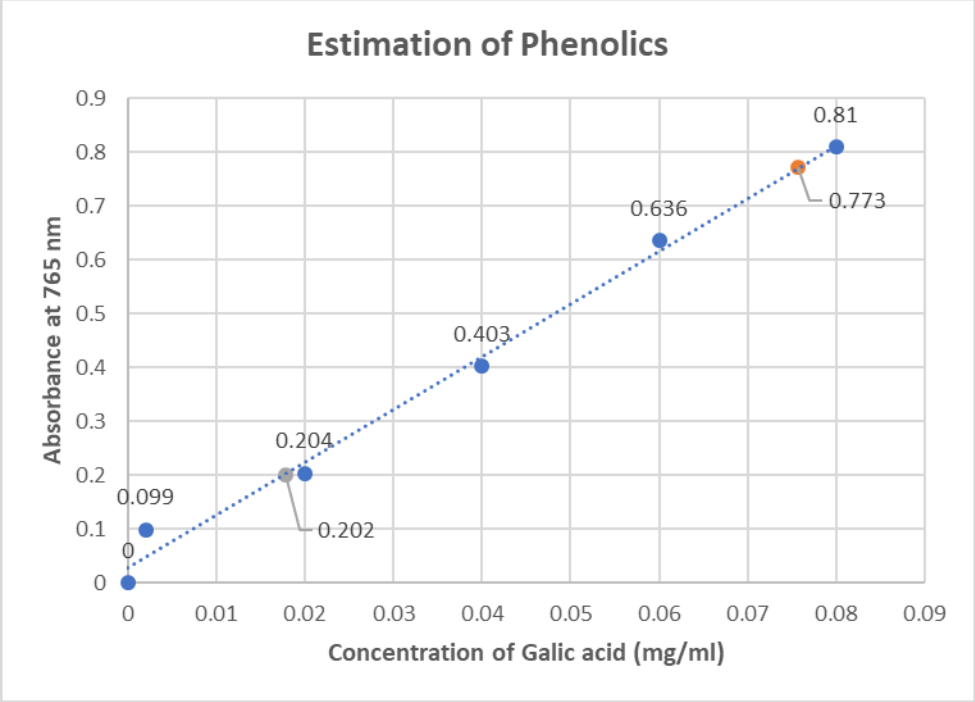
Table 2: Observation of Phenolics

Test tubes	Gallic acid (µL)	Concentration (mg\ml)	Distilled water (µL)	FC reagent (mL)	7.5%Na2CO3 (mL)	Absorbance (765 nm)
Blank	0	0	500	2.5	2	0
T1	1	0.002	499	2.5	2	0.099
T2	10	0.02	490	2.5	2	0.204
T3	20	0.04	480	2.5	2	0.403
T4	30	0.06	470	2.5	2	0.636
T5	40	0.08	460	2.5	2	0.810
EG	50	0.07564	450	2.5	2	0.773
LN	50	0.0178	450	2.5	2	0.202

Table 3: Observation of Tannin:

Test tubes	Tannic acid (µL)	Concentration (µg\ml)	Distilled water (mL)	Reagent (mL)	Absorbance (605 nm)
Blank	0	0	5	2	0
T1	100	20	4.9	2	0.043
T2	200	40	4.8	2	0.097
T3	300	60	4.7	2	0.185

T4	400	80	4.6	2	0.251
EG	500	59.19978732	4.5	2	0.548
LN	500	24.65239931	4.5	2	0.035



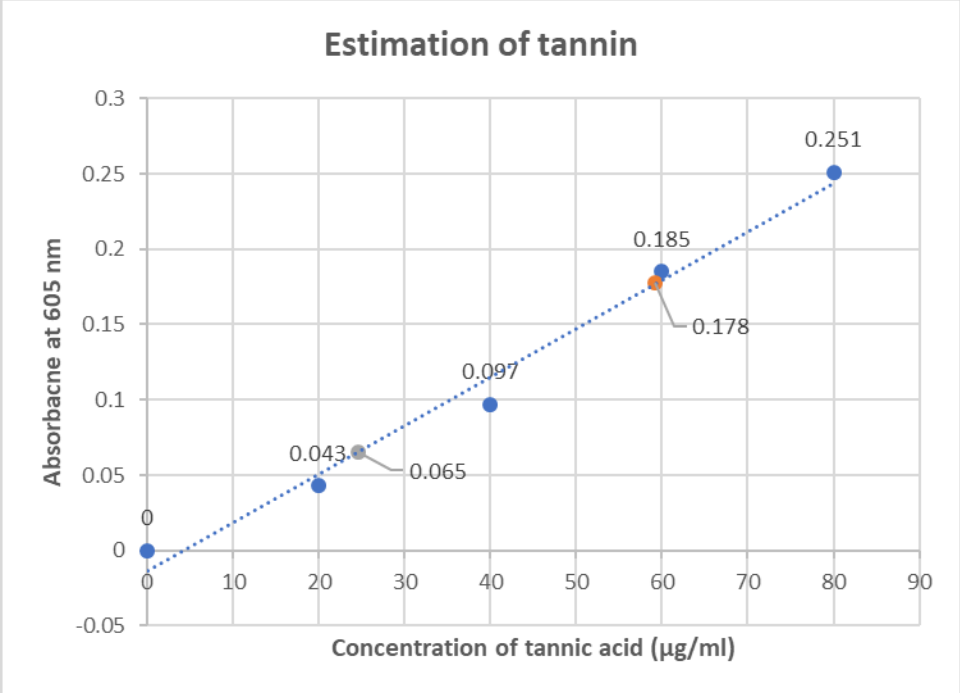


Fig1: Estimation of a. Phenolics & Tannin

Table 4: Observations of flavonoid:

Test tubes	Quercetin (mL)	Concentration (µg/ml)	Distilled water (mL)	5% NaNO2 (µL)	10% $AlCl_3$ (µL)	1M NaOH (mL)	Absorbance (510 nm)
Blank	0	0	7.4	300	300	2	0
T1	0.2	27	7.2	300	300	2	0.153
T2	0.4	54	7	300	300	2	0.219
T3	0.6	81	6.8	300	300	2	0.501
T4	0.8	102	6.6	300	300	2	0.620
T5	1	129	6.4	300	300	2	0.807
T6	1.2	156	6.2	300	300	2	1.096
EG	0.5	131.883	6.9	300	300	2	0.864
LN	0.5	83.765	6.9	300	300	2	0.523

Protein From usual curve of BSA with varying concertation given in Table 5, concentration , measured in *Eucalyptus citriodora* & *Laurus nobilis*. concentrations were found to be 0.17 as well as 0.12 g/ml, respectively. The reduction capacity of DPPH radical assessed by decrease in its absorbance at 517 nm caused by antioxidants in DPPH free radical test. extract's scavenging properties grew in lockstep with their concentrations. Table 2 shows % inhibitions of Gallic acid (as control), *Eucalyptus citriodora,* as well as *Laurus nobilis*.

Table 5: Observation of protein

Test tubes	BSA (µL)	Concentration (µg/ml)	Distilled water (µL)	Reagent 1 (mL)	Reagent 2 (µL)	Absorbance (660 nm)

Blank	0	0	1000	4.5	500	0
T1	30	0.03	970	4.5	500	0.058
T2	60	0.06	940	4.5	500	0.085
T3	120	0.12	880	4.5	500	0.151
T4	240	0.24	760	4.5	500	0.510
EG	25	0.17554	975	4.5	500	0.821
LN	25	0.12699	975	4.5	500	0.131

Table 6: RESULTS OF ANTIOXIDANT ASSAYS

Concentration (µg/ml)	**Gallic Acid**		***Eucalyptus citriodora***		***Laurus nobilis***	
	Absorbance	**% of inhibition**	**Absorbance**	**% of inhibition**	**Absorbance**	**% of inhibition**
25	0.686	61.80	0.884	50.55	0.986	45.10
30	0.612	65.92	0.752	58.12	0.921	48.71
35	0.575	67.98	0.634	64.49	0.875	51.28
40	0.468	73.94	0.529	70.54	0.758	57.79
45	0.421	76.55	0.481	73.21	0.649	63.86

Result of biochemical test

1. Motility test: Motility , shown by diffuse development away from inoculation line. Non-motile organisms can only grow in inoculation line.

2. Catalase Test: development of bubbles , deemed positive result in catalase test.

3. Oxidase test Positive responses changed bacteria's colour from violet to purple after 30 seconds. After 30 seconds, negative responses are either colourless / light pink/light purple. Delayed responses should be overlooked.

4. Sodium citrate a. Positive for citrate: growth will be apparent on slant surface, as well as medium will be deep Prussian blue.

5. Citrate negative: there will be no / just trace of growth seen. medium will not change colour; it will stay deep forest green colour of un inoculated agar. The reagent layer will stay yellow / somewhat hazy if culture indole negative. If organism urease negative, culture media will stay yellowish in colour.

Table 7: Biochemical Result

Bacteria name	**Mtl**	**Cat**	**oxi**	**citr**	**ind**	**urea**
S. aureus	+	+	-	+	-	+
E. coli	+	+	-	-	+	-
Klebseilla pneumonia	-	+	-	+	-	+

ANTIBIOTIC SENSITIVITY RESULT:

From the disc diffusion test, antibiotic sensitivity were found and calculated as MIC (minimum inhibitory concentration). Ciprofloxacin used as positive control & sterile distilled water used as negative control for the bacterial strains of *E.coli, S. aureus & K. pneumonia.*

Table 8: Result of Antibiotic sensitivity

Bacteria name	**+VE control (MIC)**	**-VE control (MIC)**	**EG extract (MIC)**	**LN extract (MIC)**
S. aureus	28 mm	No inhibition	14 mm	8 mm
E. coli	30 mm	No inhibition	20 mm	7 mm

K. pneumonia	33 mm	No inhibition	13 mm	No inhibition

EVALUATION OF HERBAL TOOTHPASTE: Herbal toothpaste has been formulated; below mentioned results found:

Evaluation of Toothpaste Physical Examination

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

Conclusion:

For majority of world's population, medicinal plants constitute most significant source of life-saving medications. Drugs, scents, pigments, food additives & insecticides are all examples of plant secondary metabolites. Phytochemical, antioxidant & antibacterial properties of Eucalyptus citriodora as well as Laurus nobilis L. were investigated in this research. These bioactivities are discovered to be different in both plants. The findings of research imply that medicinal plants may be employed in polyherbal formulations since they have antibacterial qualities as well as may be employed as antimicrobial agents & formulation of herbal toothpaste to prevent different dental illnesses caused by oral infections.

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