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Phytochemical screening, Antioxidant Activity and and Antimicrobial Suceptibility of *Camellia Sinensis* Leaf Extract against Oral Pathogen

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Abstract – In recent years, researchers have looked at the discovery of new alternative sources of antimicrobial agents, especially from plant sources. Camellia sinensis has served humanity as the source of medicinal agents from the very beginning. Phytochemical agents present in the leaves to mark the compounds responsible for the antimicrobial activity. Phytochemical screening of plant leaves reveals the presence of saponin, alkaloids, flavonoids, steroids, phenols, tannins and glycosides. Various leaf extracts of camelia sinensis were prepared and their antimicrobial activity was evaluated against the Gram-positive and gram negative bacterial strains if disc-diffusion method was done. Antimicrobial activity of many extracts of camelia sinensis leaves was created in an attempt to develop a new drug drug of natural origin for the treatment of pathogenic microbes.

Keywords: Camellia Sinensis Leaves, Phytochemical, Antioxidant, Antimicrobial Activity

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INTRODUCTION

Therapeutic use of herbs is as old as human civilization and has evolved with it. Most of the people of this planet are still dependent on their indigenous medicine and use herbal medicines. Indian and Chinese medical systems are well established, with some written records some 3000 years old. The discovery of medicinal plants medicines provides new and important links against many medicinal purposes, such as cancer, malaria, cardiovascular diseases and neurological disorders. According to the World Health Organization (WHO) report, 80% of the world's population depends primarily on conventional medicine, including the use of plant extracts or its active principle (World Health Organization, 1993).

Phytochemicals are chemical substances derived from plants and this term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical Detection Test is a simple, fast and economical process that provides researchers with an important tool for rapid response and analysis of bioactive compounds for different types of phytochemicals (Sassidharan et al., 2011).

Tea plant, *C. Sinensis* is a member of the Thei Family, and its leaves produce black, oolong and green tea. There are two varieties of tea, *C. Sinensis* variant sinensis and *C. sinensis* var Asmica (Weisborg, 1997, Dufresne and Fernworth, 2000, Wang et al., 2000, Woo and Wei, 2002, Schmidt et al., 2005). C. Signs var. The Chinese tea is known to be widely used in the sinensis, China, Japan and Taiwan, while C. Sinensis variant Assamika (known as Assam tea) is mainly involved in South and Southeast Asia, Malaysia and, more recently, Australia (Chan et al., 2007). It is a shrub or evergreen tree which is often planted in highlands of 5,000 to 10,000 plants and can grow up to 20-30 feet, but usually it is cut to 2-5 feet for cultivation. . It is grown in a wide range of latitudes ranging from 45oN to 30oS and 150oE to 60oW (Mudau et al., 2006). It grows best in the tropical and sub-tropical areas, with higher humidity, substantial rainfall and slightly acid soil, from the sea level to high mountains (Chan et al., 2007). In tropical countries, tea leaves are cut throughout the year, whereas in temperate countries crop is seasonal. To make good quality tea, the two smallest leaves and terminal buds are removed (Budau, et al., 2006, Chan et al., 2007). The leaves are dark green, alternate and with oval, serrated edges, and the flowers are white, fragrant, and appear in groups or individually. There is a fresh, slightly bitter, astringent flavor in it.

Tea is the oldest known drug. It was taken 5000 years ago in China for its stimulant and detoxifying properties in the elimination of alcohol and toxic substances, to improve blood and urine flow, to

remove joint pain and to improve the resistance of diseases (Ballantin *et al*., 1997).

Green Tea Ketchin has shown antibacterial activity against village-positive and village-negative bacteria that can be harmful to humans. Tea extract Staphylococcus aureus, S. Prevent antic lasers such as epidermis and plasomonas syngloides (Dufresne and Fernworth, 2000, 2001). Black and green tea extract can also kill the heliobacter pylori related to gastric, peptic and duodenal ulcer diseases. Tea polyphenols can selectively prevent the development of clostridia and promote the development of successful bacteria in the human large intestine. The balance of bacteria in intestinal microflora can be important for the prevention of colon cancer (Dicker and Heschelic, 1994).

Looking at this, interest in herbal medicines and naturopathy is experiencing renaissance today. Current research is based on therapeutic bioactive molecules of tea leaves responsible for the use of herbs in conventional systems and antimicrobial activity against oral and intestinal flora.

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METHODOLOGY:

Raw Materials and Sample Preparation:

The quality of an extraction is influenced by many factors, such as the solvent used for the connection between parts of the plant, extraction, extraction process and plant and solvent used to be used as an early use of the material. From the lab scale to the pilot scale, all the parameters are optimized and controlled during the extraction. Soluble plants separate metabolites through extraction techniques

Fig. 1: Tea Leaf Powder

Selection of solvents (Wichowski et al., 1908). Coffee content of coffee seeds and tea leaves was collected in January 2013 in the local market of Assam.

EXTRACTION:

The plant material was washed with tap water, cut into pieces, the air was dried and the grinding machine was turned into fine powder. Quantity of 100 grams of dry powder of tea leaf powder, individually extracted with individual individual solvents i.e. Chloroform, ethanol and water respectively using Soxhlet. The mixture with acous was due to the extracts obtained for a week. After that, extract was dissolved in a water bath at 50 degree Celsius to obtain crude oil for antioxidant assay, phytochemical analysis, determination of bioactive compounds, antimicrobial sensitivity (Gupta et al., 2011).

PHYTOCHEMICAL ANALYSIS:

Phytochemical analysis is an important process for estimating a particular chemical compound. Phytochemical analysis was done prior to the help of various specific methods for each test. All phytochemical analysis (test) (tea leaves) was used as a reference book by using Cocata (Practical Khandelwal 2008) Pharmacognosis. The phytochemical test that was done has been mentioned in Table 1

Test for carbohydrate:

A) Mobil testing:

Take 2-3 ml aqueous extracts, add some drops of α-napthol solution into alcohol. Shake and add abbreviations H_2SO_4 from the test tube. A violet ring will appear at the junction of two liquid substances.

B) Pantos Chinese test:

Mix equal quantity of test solution and HCl and heat it again. Add Foroglucinol Crystal The solution will appear in red.

C) Hexas Chinese test:

1) Tolen'foroglycylol test for galactose:

Mix 2-3ml Concert HCl and 4 mL 0.5% of phloroglucinol and then add 1.2 ml of test solution and then heat. The solution appears yellow or red.

2) Cobalt chloride test:

3ml Test solution with 2 ml Cobalt chloride spray and cool the solution and then add a few drops of the NAOH solution. The solution appears green blue.

D) Testing of non-diminishing polysaccharides (starch):

1) iodine test: mix 3 ml A few drops of test solution and diluted iodine solution. The solution will appear in blue color.

2) Tannic acid test for starch: Add 3 ml of test solution and then treat with 20% tannic

acid test solution. The solution will build PPT.

Protein Testing:

- urine test: 3ml Some drops of test solution + 4% NaOH and 1% CaSO4 solution Solution violet will appear.
- Million Test: 2ml The solution and the million reagents will blend properly. It gives the color of red brick.
- Zantho protein test: 3 ml test solution and 1 ml. Conc. H2SO4 will mix PPT white was formed
- Sulfur-containing protein test: Take 5 ml. Mix 2 ml of 40% NaOH and 10% lead acetate (2 drops) in the test solution and then boil this solution mixture. A black or brown color will appear.

Amino Acid Testing:

- Systeine test: Take 5ml. Add a few drops of solution, 40% NaOH and 10% lead acetate solution and then boil. Black PPT lead will become sulphate.

Steroids testing:

- Salkoviraktion:

Take 2 ml of solution to remove 2 ml. Chloroform and 2ml Hide Shake H2SO4 and well The chloroform layer appears red and green-yellow fluorescence will be in the acid layer.

Glycoside test:

Deoxygenian Test: Slow 2 ml extracts and add one drop glacial acetic acid and 1 drop of 5% FALX3 and 2 drops of H 2 SOS 4 concentration. A reddish brown color will appear at the junction of two liquid layers and the top layer will have a blue green color.

Anthraquinone glucoside test

- Borntrusters test for anthraquinone glucoside: Take 2 ml. Dilute the solution. H2SO4 boiled and cooled after cooling. The same amount of benzene or chloroform is well filtered and stirred. Separate the organic solvent in addition to ammonia and the ammonia layer will appear pink or red.
- C. Modified boratrez test for glycosides: Take 2 ml. Remove the solution 5% FeCl3 and add 5 ml. heart. HCl After cooling the water bath and adding benzene or any organic solvent it was boiled for 5 minutes boiling. Stir well Separate the organic solvent layer and then add the same amount of diluted ammonia. Ammonium layer can be converted to pink red.

Flavonoid test

- Take 2 ml of the test solution and then add a small amount of low acidity of lead acetate (10%). The solution will make ppt yellow color.
- Addition of increasing amount of sodium hydroxide to residues. Yellow color of the formed solution, which dissolves after the addition of acid.

Alkaline test

- Mayer Trial: Take 2- 3 ml. Filtered extraction solution with a few drops of mayor reagent. The solution will build PPT.
- Wagonar Test: Take 2-3 ml. Filtered extracts solution with some drops of Wagners reagent. The solution will be red brown PPT.

Tannic acid and phenol compound test:

2-3ml Add some drops to the next transfection for aqueous or fluffy extracts.

- 5% FeCl3 solution. The slurry will appear in dark blue color.
- Lead acetate solution (10%). The solution will build white PPT.
- Gelatin Solution (10%). The solution will build white PPT.
- Acetic acid solution. The solution will appear red.
- Slim iodine solution. The solution will appear in red.
- Slim potassium permanganate solution. Solutions will abandon your color.

Organic acid test:

- **Oxalic acid confirmation test: -**

Ml 2ml Mix a few drops of 5% lead acetate test solution. PPT white was formed

Ml 2ml Add a few drops of test solution 1% KMnO4 and diluted. H2SO4. The color of KMnO4 disappears.

- **Malic acid confirmation:**

2-3 ml. The test solution adds 2-3 drops of 40% FeCl3. The solution will look yellow.

Test for Inorganic Acid:

- **Sulfate Test**

Returns the white ppt with the reagent with lead acetate. Which will be soluble in NaOH solution

Chloride test:

5-7 ml 3-5 ml filtering solution to remove the vegetable. Lead the acetate solution. Give White PPT Soluble heat is seen in hot water.

Carbonate test:

- With diluted acid release CO2 gas.
- Mercuric chloride solution, the slurry will make a red-brown PPT.

Nitrate test: The solution of plant extracts does not produce brown color with the solution of ferrous sulphate, but if sulfuric acid is in the mix of two fluid in brown color.

Coumarin glycoside test:

- This fragrant odor is mixed with Coumaringlycosides.
- Alcohol extracts, when it becomes alkaline, shows blue or green fluorescence.
- Take powdered powder with dry powder in the test tube. Cover the test tube with filter paper soaked in the diluted NaOH. After exposing the UV light to the filter paper, place it in a water bath. Now it is yellow green fluorescence.

BIOACTIVE COMPOUNDS:

1) Flavonoid content:

Flavonoid content of isolated crude (tea leaves and coffee seeds) was determined with this method (Gia et al., 1999). Take a clean test tube and add 0.5 ml of the specimen (extract) to 1.25 ml of distilled water. Then 0.075 ml of 5% sodium nitrite solution was added and allowed to stand for 5 minutes. 0.15 ml of 10% aluminum chloride was added, after 6 minutes, 0.5 ml of 1.0 ml sodium hydroxide was added and the mixture was diluted with 0.275 ml of distilled water. The absorption of the mixture was immediately measured at 510 nm. Flavonoid content was expressed in the form of sample mg of equivalent catechins / g and the same process was done with extract of tea leaves and coffee seeds.

2) Total phenolic content:

With modest modification, the ability of total phenol material was determined using the method. The total phenolic content of isolated crude (fruit powder and leaves) was determined by the method described by (singleton et al., 1965). The sample of 1.0 ml was mixed with Falin and 1.0 ml of Sioltecu phenol reagent. After 3 minutes, 1.0 ml saturated Na2 CO 3 (~ 35%) was added to the mixture of 2 3 and the distilled water was added to 10 ml. The reaction was maintained in the dark for 90 minutes, under the UV-Vis Spectrophotometer was observed on the absorption of 760 nm. Gallic acid was used as a standard with a concentration of 200 ppm at 1,000 ppm. As a standard, a calibration curve was created with different concentrations of catechol (0.01 to 0.1 mm). The results were expressed in milligrams of catechol / g extract and the same procedure was taken with the removal of tea leaves and coffee seeds.

3) Free radical scavenging activity:

DPPH Free Radical Scavenging Activity

The activities of free radical upliftment of these compounds were proved by their ability to reduce the stable radical DPPH. Evaluation of antioxidant activity using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was done by this method (Benzie et al., 1996). Sample extracts and standards (BHA and ascorbic acid) were prepared at different concentrations (200-1000 ppm) and mixed with ethanolic DPP solution in the concentration of 0.04 mg / mL. After standing in the dark for 20 minutes, using the UV-Vis Spectrophotometer, the mixture against the ethanol as a target was measured at 517 nm and the same process was taken with the removal of tea leaves and coffee seeds.

The result obtained was calculated using formula:

Scavenging Activity AA (%) = (Abs control – Abs sample) / Abs control x 100

Nitric oxide radical scavenging activity:

The process is based on the principle that the body produces sodium nitroprusside nitric oxide in the aqueous solution on pH oxygen ions can be estimated using nitrite intensely Griess reagent, with the exchange of information. Nitric oxide powders are complete with oxygen, reducing the production of nitrite ions. Plant removal was dissolved in distilled water for this quantitation. The incubate with sodium nitroprusside (5 mm) phosphate buffer were incubate at different concentrations (100-400 .mu.g / mL) and tubes for

methanol extract in saline (0.025m, pH 7.4) were incubate with 3 hours. Without experimenting compounds but with an equal amount of buffer control experiment was demonstrated positively. After 3 hours, incubate samples were diluted by 1 ml of Griess reagents. The absorption of color developed during diazotization with later coupling with nitrite sulfanilamide and naphthylethylenediamine hydrochloride was observed at 550 nm in spectrophotometer. The same process was compared with ascorbic acid, with standard methanol extract performed. He said that compared to the standard graphic formula and the% refusal calculation from the graph (Singh et al., 2012).

$$\text{Nitric Oxide Scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where, $A_{control}$ = Absorbance of control reaction

A_{test} = Absorbance in the presence of the samples of extract.

Total antioxidant capacity assay:

The assessment of the total antioxidant capacity was determined by the Phosphobolibdenum Method (Preto et al., 1999). 0.3 ml of extracts and sub-parts were mixed in ethanol; ascorbic acid was used separately with standard (5 to 200 g / mL) and 3 ml reagent mixture with white (ethanol) and 95 degrees Celsius But incubation was done. 90 min After cooling the room temperature, the absorption of each sample was measured against the vacuum at 695 nm. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalent to ascorbic acid or gelic acid 16. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and is computed by the following equation:

$$A = (c \times v)/m$$

Where,

A = total content of antioxidant compounds, mg/g plant extract, in Ascorbic Acid Equivalent,

c = the concentration of Ascorbic acid established from the calibration curve, mg/ml,

V = the volume of extract (ml), and m = the weight of crude plant extract (g).

Thin Layer Chromatography

Thin layer chromatography (TLC) is a method of analysis in which the stable phase, a micro-split concrete, extends into a thin layer on a rigid support plate; And in the mobile stage, a liquid is allowed to move to the surface of the plate. Although the ablation capacity obtained with gas chromatography or high pressure fluids cannot be obtained by this method, but they have the advantages of speed, versatility and simplicity.

The solvent system was inserted to a depth of 0.5 cm in a rectangular chromatographic glass chamber. The chamber was lined with a piece of filter paper to ensure adequate saturation. Extract spot was applied to silica gel g plate with the help of a capillary tube. The distance between the two points is about 2.0 cm. Applied spots were dried at room temperature and the plate was gently placed inside the glass chamber. The angle of the plate with vertical remains approximately 15 °. The chromatogram was developed until the solvent front had reached almost 10.0 cm. The plate was removed and the solvent was marked in front. Plate was dried at room temperature and inspected under UV light or sprayed with specific identification reagent. Colored spots were marked and the RF value of each separate component was calculated.

It was observed during all TLC studies that the derivation of the plates did not give a good resolution with different detection reagents and the number of spots was also lower when UV light was seen, hence the selection of UV light detection was selected. Full test of TLC plates.

***Antibacterial Activity of Extract Coriander* leaf & seed:**

For antimicrobial activity, a microbial sample of various oral flora was separated using Swab method and spread on special media plates and then incubation at 37 degree Celsius. After bacterial culture, isolates stained with gram stain and a 100x compound microscope were observed. According to morphometric characterization, 5 different bacterial isolates are maintained in the following manner

Microbial Strains:

- *S.mutans*
- *E .fecalis*
- *E.coli*
- *S.aureus*
- *P.aeruginosa*

Disc diffusion method:

Disk Spreading Method (Kirby Bowr et al., 1966) was used to test the antibacterial activity of extracts against 5 bacterial species. For this purpose, cultivate liquid broth overnight. Put the OD on 660 nm (1 x 10 6) Spread the culture on NAM agar medium Whitman no. Place 1 filter paper in 6 mm of different concentrations of plant extracts of 6

mm. 75 mg/dilution ml (25,50,75,100%). Incubate at 37 ° C for 8 hours. Measure the area of negation in the zonal scale (hi-media) in mm. (Dougher et al 2008).

Phytochemical investigation

1. Extraction Yield:-

Extraction Yield for Leaf & Seed Powder was found to be higher for Aqueous Extract. The total % of yield for Soxhlet (solvent-300ml) was (Soxhlet- 10 %) respectively.

Table.1: The total % of yield was as follow

S. No.	Extraction	% yield
1.	Methanolic Extract of Tea Leaves	28.32%
2.	Aqueous Extract of Leaf	49%
3.	Chloroform Extract of Leaf	19%

2. Phytochemical investigation

The result of phytochemical screening reveals alkaloids, steroids and saponins were found to be present in aqueous extracts of Leaf & Seed Powder of Tea leaves and Coffee seeds (Table 2)

Table.2: Phytochemical screening of methanolic extract of tea leaves

S. No.	Phytochemical test	Tea leaves extract
1.	Carbohydrate	Positive
2.	Protein	Negative
3.	Amino acid	Negative
4.	Steroids	Positive
5.	Glycosides	Positive
6.	Anthraquinone glycosides	Negative
7.	Flavonoids	Positive
8.	Alkaloids	Negative
9.	Test for tannic acid and phenolic compounds 1. 5% $feCl_3$ solution 2. Lead acetate solution 3. Gelatin solution 4. Acetic acid solution 5. Dil. Iodine solution 6. Dil. Potassium per magnate	 Positive Negative Negative Positive Negetive Negative
10.	Organic acid	Negative
11.	Inorganic acid	Negative
12.	Chloride	Negative
13.	Carbonate	Negative
14.	Nitrate	Positive
15.	Coumirin glycosides	Negative

Table.3: Phytochemical screening of chloroform extract of tea leaves

S. No.	Phytochemical test	Tea leaves extract
1.	Carbohydrate	Positive
2.	Protein	Negative
3.	Amino acid	Negative
4.	Steroids	Positive
5.	Glycosides	Negative
6.	Anthraquinone glycosides	Negative
7.	Flavonoids	Positive
8.	Alkaloids	Positive
9.	Test for tannic acid and phenolic compounds 1. 5% $feCl_3$ solution 2. Lead acetate solution 3. Gelatin solution 4. Acetic acid solution 5. Dil. Iodine solution 6. Dil. Potassium per magnate	 Positive Negative Negative Positive Positive Negative
10.	Organic acid	Negative
11.	Inorganic acid	Negative
12.	Chloride	Negative
13.	Carbonate	Negative
14.	Nitrate	Positive
15.	Coumirin glycosides	Negative

Table.4: Phytochemical screening of Aqua's extract of tea leaves

S. No.	Phytochemical test	Tea leaves extract
1.	Carbohydrate	Negative
2.	Protein	Negative
3.	Amino acid	Negative
4.	Steroids	Negative
5.	Glycosides	Negative
6.	Anthraquinone glycosides	Negative
7.	Flavonoids	Positive
8.	Alkaloids	Positive
9.	Test for tannic acid and phenolic compounds 1. 5% $feCl_3$ solution 2. Lead acetate solution 3. Gelatin solution 4. Acetic acid solution 5. Dil. Iodine solution 6. Dil. Potassium per magnate	 Negative Negative Negative Negative Negative Negative
10.	Organic acid	Negative
11.	Inorganic acid	Negative
12.	Chloride	Negative
13.	Carbonate	Negative
14.	Nitrate	Negative
15.	Coumirin glycosides	Negative

Qualitative analysis by TLC:

Qualitative analysis had done by TLC (thin layer chromatography) using Ethyl acetate: Formic acid: Water (6.5:1.5:2) solvent system. RF value of produced content 1.06 and 3.3 in crude of leaf and RF value of produced content was 1.19 and 1.42 in produced crude in seed.

Fig.2: TLC of Tea Leaf extract observed under U.V. Light

Total phenolic (TPC) assay:

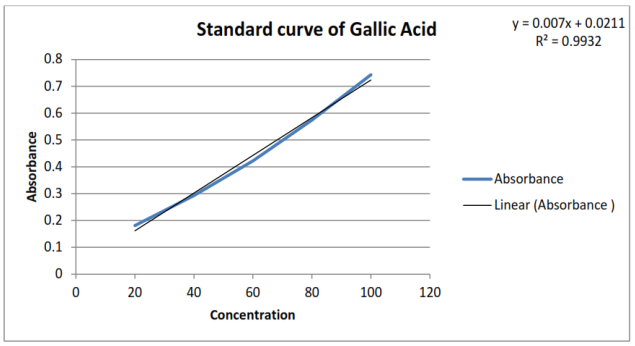

Fig. 3: Standard Curve of Gallic Acid

Table.5: Total Phenol and Flavonoid Levels of Selected Coffee Bean Varieties

QUANTITATIVE ANALYSIS	Tea
TOTAL PHENOLS (mg of TAE/serving)	0.03±0.05

The results of the Folin-Ciocalteu total phenol assay with aqua's extract were reported in Table.5. Sample of Tea leaf was show phenol compound. The Total Phenolic content of Tea leaves contained the considerable amount show in table.5

Total flavonoid (TFC) assay:

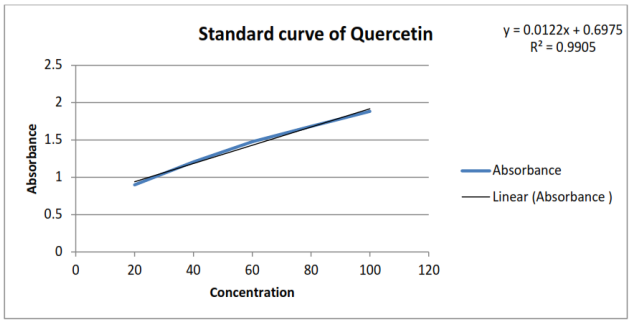

Fig. 4: Standard Curve of Quercetin

Table.6: Total Flavonoides in Levels of Selected Tea leaf extract

QUANTITATIVE ANALYSIS	Tea
TOTAL FLAVONOIDS (mg of CE /serving)	0.02±0.09

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The values of flavonoid content was observed in tea leaves 0.02±0.09mg/gm (Table 6).

Table.10: Total Antioxidant scavenging activity

S. No.	Concentration	Standard Ascorbic Acid	Value (Leaf)	Value (Seed)
1.	20 µg	0.652	0.678	0.489
2.	40 µg	0.692	0.565	0.234
3.	60 µg	0.723	1.202	0.214
4.	80 µg	0.793	0.215	0.745
5.	100 µg	0.813	0.577	0.833

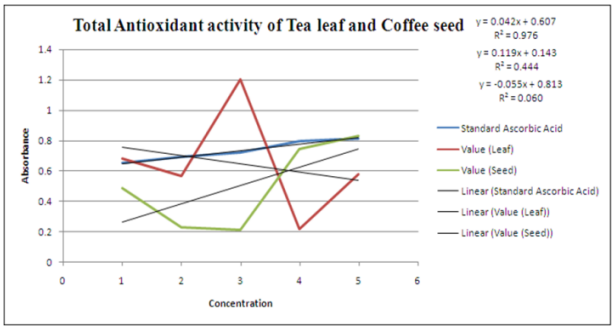

Fig.5: Total Antioxidant scavenging Activity.

Total antioxidant capacity equivalent of ascorbic acid was 0.996 mg/g of extract. Concentration ranging from 20-100 µg /ml of the aqueous extract of Tea Leaves was tested for their antioxidant activity in different in vitro assay. It was observed that the free radical scavenged by the extract was show in table-3.

Table.11: DPPH scavenging activity

S. No.	Concentration	Standard Ascorbic acid	Tea	Coffee
1.	20 µg	0.994±0.40	0.224±0.69	0.432±0.30
2.	40 µg	1.251±0.61	1.266±0.19	0.472±0.67
3.	60 µg	1.622±0.24	0.301±0.35	1.599±0.98
4.	80 µg	1.174±0.50	0.065±0.22	0.092±0.63
5.	100µg	1.977±0.52	1.789±0.61	1.162±0.19

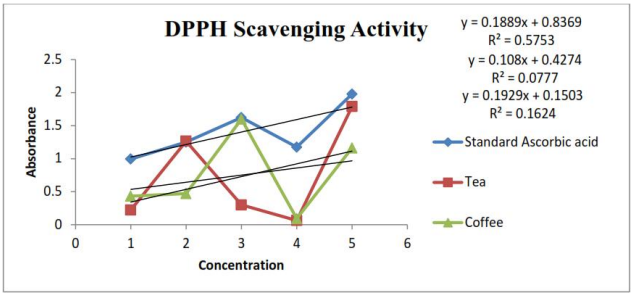

Fig .6: DPPH scavenging activity

DPPH scavenging activity equivalent of ascorbic acid was 0.994 mg/g of extract. Concentration ranging from 20-100 µg /ml of the methanolic extract of Tea leaf and Coffee seed was tested for their antioxidant activity in different in vitro assay. It

was observed that the free radical scavenged by the extract was show in table-7.

Table.12: Nitrous oxide scavenging activity

S. No.	Concentration	Standard ascorbic acid	Tea	Coffee
1.	20µg	0.256±0.08	0.178±0.05	0.152±0.12
2.	40 µg	0.232±0.09	0.169±0.08	0.226±0.05
3.	60 µg	0.267±0.07	0.255±0.06	0.164±0.01
4.	80 µg	0.245±0.09	0.198±0.04	0.198±0.08
5.	100 µg	0.276±0.04	0.211±0.05	0.219±0.08

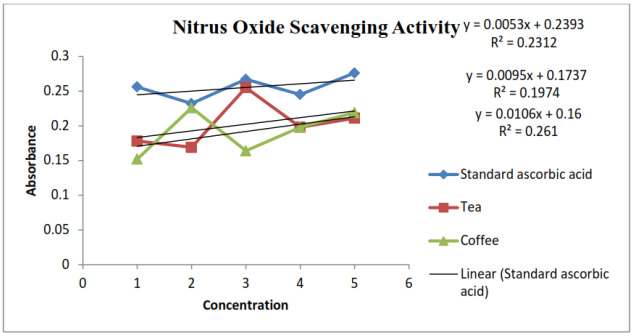

Fig.7: Nitrous oxide scavenging activity of Mangifera Indica Seed powder & Leaf

Nitrous oxide scavenging activity equivalent of ascorbic acid was 0.255mg/g of extract. Concentration ranging from 20-100 µg /ml of the methanolic extract of *M.indica* was tested for their antioxidant activity in different in vitro assay. It was observed that the free radical scavenged by the extract was show in table-8.

Table.13: Antimicrobial Susceptibility (Tea Leaf)

S. No	Sample ID	Concentration			
		25mg/ml	50mg/ml	75mg/ml	100mg/ml
1.	S. mutans	17mm	19mm	12mm	23mm
2.	E.fecalis	13mm	16mm	18mm	22mm
3.	E.coli	13mm	16mm	17mm	19mm
4.	S. aureus	12mm	14mm	20mm	22mm
5.	P. aeruginosa	16mm	17mm	18mm	20mm

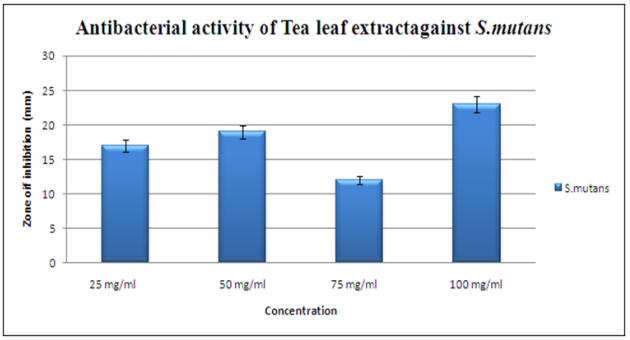

Fig.8: Antibacterial activity of Tea leaf extract against *S.mutans*

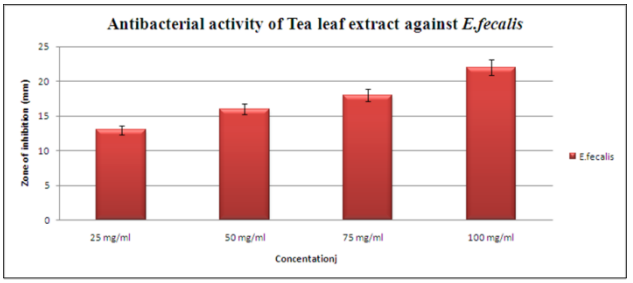

Fig.9: Antibacterial activity of Tea leaf extract against *E.fecalis*

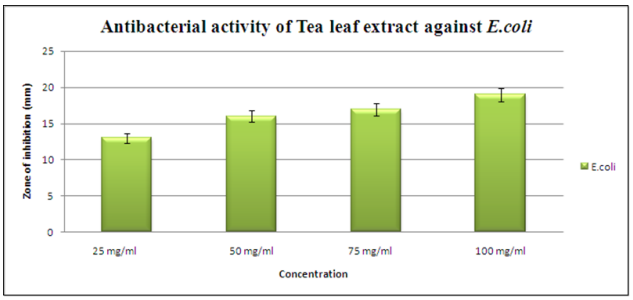

Fig.10: Antibacterial activity of Tea leaf extract against *E.coli*

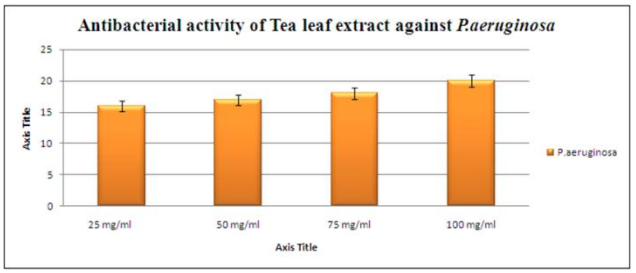

Fig.11: Antibacterial activity of Tea leaf extract against *P.aeruginosa*

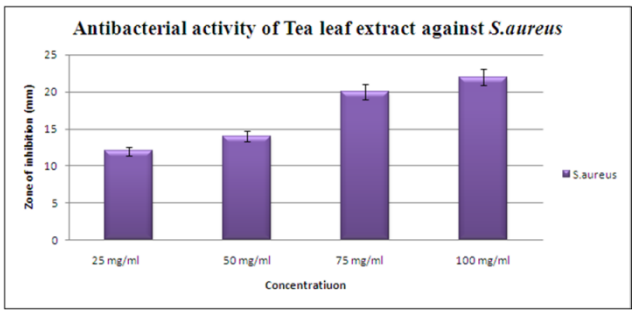

Fig.12: Antibacterial activity of Tea leaf extract against *S.aureus*

Tea leaf extract showed maximum inhibition against selected oral isolates Streptococcus mutans i.e. 23 mm, 22mm and 20mm respectively. The observations were comparable with that of other studies. The flavonoids and tannins present in the leaf are responsible for their antibacterial properties [fig.8-12 and table-9].

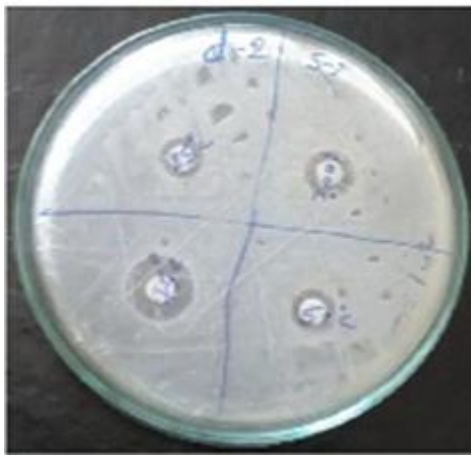

Fig. 13: *S. mutans*

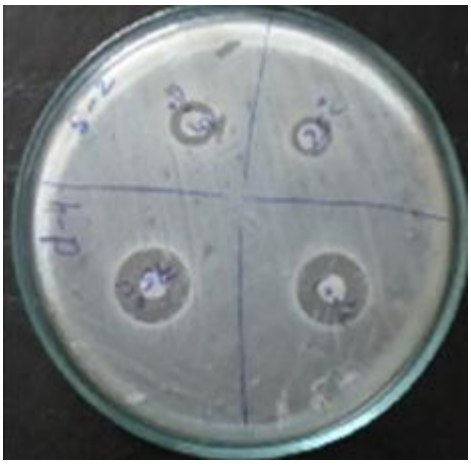

Fig. 14: *E. fecalis*

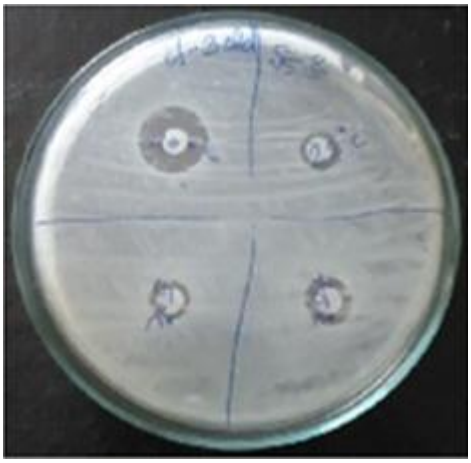

Fig. 15*: E. coli*

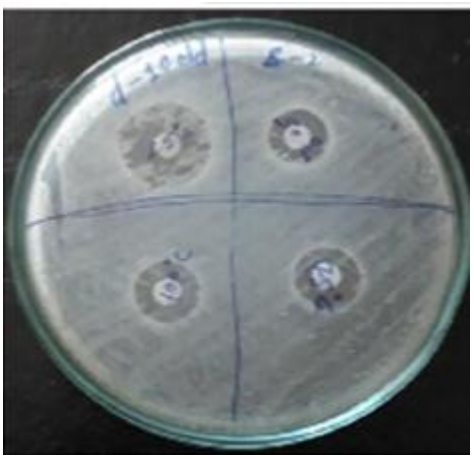

Fig. 16*: S. aureus*

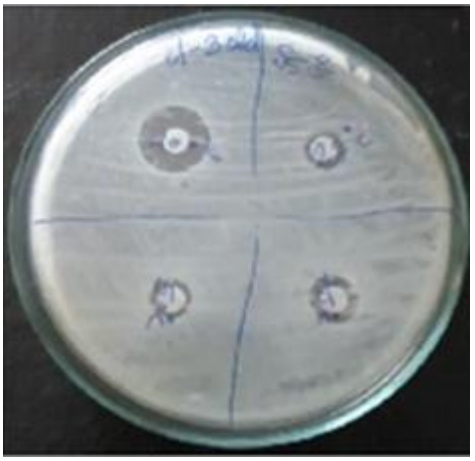

Fig. 17: *P. aeruginosa*

DISCUSSION:

India recently researched traditional herbal medicines after scientific confirmation of its effectiveness in the treatment of those conditions, for which they were traditionally determined. Current research has discovered the use of spatial tea leaf plant throughout India for the treatment of infectious diseases.

The antimicrobial examination showed interesting activities in all concentrations: all extracts were active. Plant extracts are generally a raw mixture of active and non-active compounds, and the areas of their inhibition should be interpreted accordingly.

We note that the area of prohibition of less than 50 mg / ml should be interpreted as a strong antibacterial capacity. The area of prohibition received in this study is considered to be important for plants and even pure extracts, within the bounds. The study clarified a complex ethnographic picture in terms of extraction. First of all, the results showed the importance of extraction, the extraction method can also change the results. For antimicrobial activity, plant extracts were active against Gram-positive and Gram-negative bacteria. The activity depends on the stress of the bacteria,

the nature of the plant, its maturation status and the nature of the extraction.

The antimicrobial activity of the removal of camelia sinensis has been demonstrated in other studies, but the antibacterial effects of this plant have not been studied against oral pathogens (Shapana et al., 2010, Meghashish et al, 2009). The current study supports the opinion that Camellia sinuses may be useful in antioxidant agents against oral pathogens. The findings of this study suggest that Camellia sinensis S. Mutants, E. Faklis, E. Koli, S. Aureus, p. Can stop the development of erginosa.

All verbal bacteria in the ethanolic extracts of Camellia sinensis, especially S. Mutten, E. Faklis, E. Koli, S. Aureus, p. There was a promising MIC value against Ereuginosa. Although in some studies, it has been reported that many bacteria such as S. Orrios, E., in Camellia sinensis extract. Falcis and E. Antioxidant activity is against coli, but oral pathogens such as A. There are some studies on Viscosus and S. Sangis (Nirmala et al., 2011).

In the current study, the ethnolic extracts of this plant were found in E. Exhibit the highest MIC value against coli, therefore, the germicidal activity of camelia sinensis against gm-positive bacteria was probably more than negative negative bacteria.

s. Mutations, the micro-organisms against which our extracts against them were most active, should be related to the cause of the disease by that microorganism. Activity cannot be used in the same family (or their absence) of phytoochemicals. Alkaloids, flavonoids and phenolics usually have antimicrobial properties; Therefore, its presence in the roots can explain the activity of this organ. However, alkaloid cannot be fully responsible for the activity. It is known that flavonoids (found in all seed extracts) are synthesized by plants in response to a microbial infection (fogalani et al., 2005); Therefore, its ability to in vitro antimicrobial efficacy against a wide range of microorganisms should not be surprising. It can be said that the majority of phytochemicals known by qualitative chemical analysis and work are known in other plant species, which contain antimicrobial medicinal activity.

Regarding antibacterial activity, the current study showed an important area of prohibition for the extract of raw plant against all strains. s. The powerful activity against mutations welcomes the infection especially due to the frequency of bacteria, and most bacterial clinical infections are due to this stress. s. Equally strong activity against aerus opens the possibility of using alone or using a combination of other antibacterial drugs.

As for antimicrobial activity, antibacterial activity can be attributed, possibly in combination, during the chemical selection of extracts, many phytochemicals are detected and harm the cell membrane, which filtration of cellular material And eventually the example, death of microorganisms.

CONCLUSION:

With all these broad spectrum antibacterial properties, tea leaves. It can be considered an effective antimicrobial agent for the treatment of infectious diseases. This plant showed activity against some of the bacteria prevailing in oral infections. The study scientifically supported the ethno pharmacological use of the plant as an antimicrobial agent and could explain some of the forms seen in ethnopharmaceutical preparation methods. Therefore, the use of this plant as an antimicrobial agent is valid from the results obtained in this work. More studies are being done to identify the chemical compounds of these antimicrobial extracts.

Extra study is required for the separation of active ingredients for the removal of tea leaves and to confirm the mechanism of action of extracts that can be responsible for the antimicrobial activity of extracts. A detailed study on these selections is also a parameter that should be included in later studies.

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