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Clove Based Herbal Tea: Development, Phytochemical Analysis and Evaluation of Antimicrobial Property

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Abstract:

Tea, one of the most commonly consumed rejuvenating beverages all across the world, had recently gained immense attention in the field of research with a keen focus on the exploitation of its medicinal value and therapeutic potential as a form of complementary and alternative medicine (CAM). In this backdrop, a very popular natural ingredient clove is selected for blending with a few varieties of green and black tea in different quantities to formulate herbal tea that has excellent health-promoting therapeutic activity. Phytochemical and antioxidant properties were analysed along with their antimicrobial activity.

The results highlighted that the herbal preparation of tea + clove, when mixed in very high concentration (1000mg), contained the highest amount of phenolic content mg/GAE (1007.25 ± 1.75) , flavonoid content mg/CE (158.17 ± 2.14) free radical scavenging capacity in terms of % inhibition (96.81 ± 0.16) along with enhanced antimicrobial property with respect to the others. Hence, we can conclude that drinking tea mixed with clove can surely be a choice of medicinal beverage.

Keywords: Antioxidants, Flavonoids and Polyphenolics, Antimicrobial, Tea, Clove

INTRODUCTION:

Tea is a refreshing and aromatic beverage prepared by steeping the leaves of *Camellia sinensis* in hot water. Based upon how tea is produced and processed, it can be classified as – black (fermented) tea and green (non-fermented) tea. While green tea leaves are steamed after drying, black tea leaves and buds are oxidized after drying.[1] [2]Tea is particularly rich in bioactive compounds like polyphenols, flavonoids, and antioxidants. Besides its exceptional aroma and rejuvenating taste, tea has gained popularity as a drink because of its potential health-enhancing activities.[3] Herbal tea is a recipe of low-cost tea mixed with several naturally occurring compounds such as herbs and spices like clove, ginger, citrus rind, turmeric, etc. Of late, herbal preparations of tea are being explored and exploited for its medicinal value and therapeutic potential as a form of complementary and alternative medicine (CAM). In this backdrop, the different health-promoting and therapeutic activities of some teas, and widely used culinary herbs and spices were studied.

Cloves (*Syzygium aromaticum*), are small, reddish-brown aromatic flower buds of a tropical evergreen tree of the family Myrtaceae. They behold sharp, biting flavours and strong aroma. Native to the Maluku Islands (or the Moluccas) in Indonesia. [4] Cloves are one of the most commonly consumed dietary condiments in the world. They represented an important component of the traditional system of medicines. Eugenol, the main bioactive compound in clove, possesses great potential for pharmaceutical, cosmetic, food, and agricultural applications. [5] Clove has important medicinal properties such as it helps in digestion, reduces blood pressure, cures toothache, helps against halitosis, mouth, and throat inflammation. 14 Many scientific reports confirm the antimicrobial, antiseptic, anti-diabetic and anti-carcinogenic properties of this spice plant. [6] [7]

Based on these above scientific findings the present course of study aims at development of clove based herbal infusions of black and green tea by blending with the clove and subsequently phytochemical analysis, and deciphering their antioxidant and antimicrobial activity was done and compared with normal green and black tea infusions.

MATERIALS AND METHODS

Chemicals and Reagents Used:

Reagents used for quantitative estimation of phytochemical analysis such as total polyphenol and flavonoid content along with antioxidant studies were Gallic Acid (Merck), Catechins (Sigma), Ascorbic Acid (Sigma), Follin-ciocalteu reagent (Merck), Sodium carbonate (Merck), Sodium Hydroxide (Merck), Anhydrous Sodium Nitrite (Merck), Aluminum Chloride (Merck), DPPH (Merck), Methanol (Merck). Another set of chemicals used for the analysis of antimicrobial properties were Luria Broth (Merck), and Luria Bertini Agar (Merck). All chemicals were analytically graded. HPLC grade water was (Merck) used throughout the project.

Sample Collection:

Two green tea (Lipton Green (G1) & Dilmah Green (G2)), two black tea (Okayti Delight (B1) & Okayti Second Flush (B2)), and one of the common culinary spices clove, were purchased from a local market situated in Kolkata, West Bengal, India, during the month of December 2018.
Normal Green and Black tea were kept as control.

Sample Preparation:

After collecting all the samples, they were dried (if required), ground, sieved and processed to obtain a finely powdered sample which was stored in airtight containers for further analysis. Then the powdered tea was measured and thoroughly mixed with each of the other powdered ingredient clove in varying ratio, to obtain the desired

herbal mixtures. Then, 2g of normal tea as well as herbal tea were measured and were brewed in 100 ml hot boiling water (distilled) at 100°C for 5 minutes. The solutions so obtained were then filtered, and the clear flow-through was collected in 100 ml volumetric flasks. The volume was made up with water and used for the subsequent assays. [8] (Chatterjee S et al. 2014)

Phytochemical Analysis (Quantitative Assays)

Estimation of Total Polyphenols

The total polyphenolic content (TPC) was determined by using the Folin-Ciocalteu method with slight modifications. 1:10 Follin-Ciocalteau reagent was mixed with 7% sodium carbonate. Gallic acid was used for preparation of the standard curve. The colorimetric change was measured in a UV-VIS spectrophotometer, and the absorbance read at 765 nm. Concentration of the total polyphenol content was expressed as mg Gallic acid equivalent/cup of tea [9] *(Singleton V.L et al. 1965).*

Estimation of Total Flavonoids

Total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric assay with slight modifications. 5% Sodium nitrate were mixed with 10% Aluminium chloride. After 6 mins 1M Sodium hydroxide were added. Catechin was used as the standard for preparing the calibration curve. The colorimetric change was measured in a UV-VIS spectrophotometer, and the absorbance read at 510 nm. Concentration of the total flavonoid was expressed as mg Catechin equivalent/cup of tea [10] (*Jia et al. 1999*).

DPPH Radical Scavenging Assay

The free radical scavenging assay was performed according to the standard method (*Shen et al. 2010*) with a slight modification by using Ascorbic acid as standard. The colorimetric change was measured in a UV-VIS spectrophotometer, and the absorbance read at 517 nm against methanol used as blank. The DPPH radical scavenging activity was expressed in terms of Ascorbic acid equivalent, and the percentage of inhibition calculated by the following formula [11] (*Blois M.S et al. 1958*) :

% Inhibition of DPPH

= [(Absorbance of control –Absorbance of the sample)/ Absorbance of control] *100.

Determination of Antimicrobial Property by Kirby Bauer Disk diffusion method (Biological Assay):

Test Microorganisms:

The two bacterial strains used in the present study were obtained from Calcutta University, West Bengal:

Gram-positive bacteria: *Staphylococcus aureus*

Gram-negative bacteria: *Escherichia coli*:

Antimicrobial activity of the herbal tea was determined using the slightly modified Kirby Bauer Disk diffusion technique. The sample extracts for this assay were filtered through 0.2 µm Whatman Filter paper before use for this purpose. In 5ml of sterile Luria Broth, 100 µl of each microbe was subcultured. 20µl of test bacteria from the log phase of freshly sub-cultured tubes were spread and seeded onto pre-warmed sterile LB Agar plates. Sterile paper discs were placed onto the surface of inoculated agar plates with the help of sterile forceps. 30µl aliquots of sample extracts were then pipetted out onto the paper discs embedded on the agar surface. The plates were allowed to dry for some minutes and then incubated at 37ºC for 24hrs. Antibacterial property was expressed as the diameter of zone of inhibition (mm) produced by the extracts around the disc. All tests were carried out in triplicates [12].

Statistical Analysis

All the experimental measurements were performed in triplicate and expressed as the average ± standard deviations. The magnitude of the correlation coefficient between two variables, means, standard errors, standard deviations, and one way ANOVA were calculated by using MS Excel Software. Statistical significance was accepted at $P < 0.05$.

RESULTS

Phytochemical Analysis (Quantitative Assays):

In the present course of study aims at determination of antioxidant and antimicrobial activity of the different clove based herbal teas and compared with the unfortified black and green tea. Since antioxidant potential of herbal extract mainly depends on the concentration of total polyphenols (TPC) and flavonoids (TFC), therefore we determined their concentration based on the standard methods and correlated with their free radical scavenging activity.

Standard curve for TPC, TFC and radical scavenging activity were prepared taking Gallic Acid, Catechin and Ascorbic Acid respectively (Table:1) where R2 Value highlighted the strength and accuracy of the quantitative evaluation.

The total polyphenol content (TPC), total flavonoids content (TFC) antioxidant potential of Green tea n=2) and Black tea (n =2) was determined and compared with that of the prepared green and black tea herbal infusions respectively.

Table 1: Standard curve equation and their R2 value of different phytochemical assay

Sr. No	List of Quantitative Assay	Std. curve Equation	R^2 Value
1	Polyphenols (Gallic Acid)	Y=10.478+0.0495	0.9997
2	Flavonoids (Catechin)	Y= 4.2865x+0.004	0.9999
3	Antioxidant DPPH (Ascorbic Acid)	Y= 343.44x+0.5613	0.9997

Total Polyphenol Content (TPC) and Total Flavonoids Content (TFC):

In the present study, green tea (GT) and black tea (BT) used as control. The total polyphenol content (TPC) and **Total Flavonoids Content (TFC)** of green (G1 & G2) and black (B1 & B2) tea were determined and compared with the clove based herbal tea respectively. It is observed that with the increasing concentration of clove, TPC and TFC were also increases linearly as evident from **Fig 1 (a),(b), Fig 2 (a),(b) and Table 2,3.** In case of green tea, the decreasing order of TPC and TFC concentration is, G1 + C5 > G1+C4 > G1+C3 > G1 +C2 > G1 +C1. Statistically significant differences (P value is <0.0001) were observed among these different concentrations of clove based tea with the normal one. In case of black tea, similar kind of data was obtained. In case of black tea, the decreasing order of TPC and TFC concentration is B1 +C5 > G1 + C4 > G1+C3 > G1 +C2 > G1 +C1. This result is also shown statistically significant (P value <0.0001).

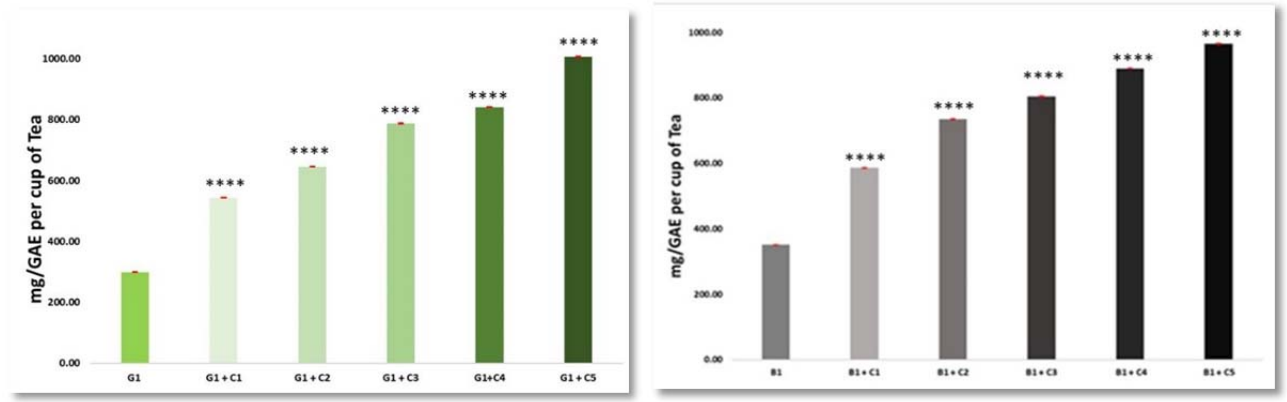

Figure: 1(a) and (b) Representative graph showing comparison of total phenolic content (TPC) of original plain green tea (G1) and black tea (B1) as control, infused with varying concentrations of clove (C1; C2; C3; C4; C5). Here ** signifies highly significant.**

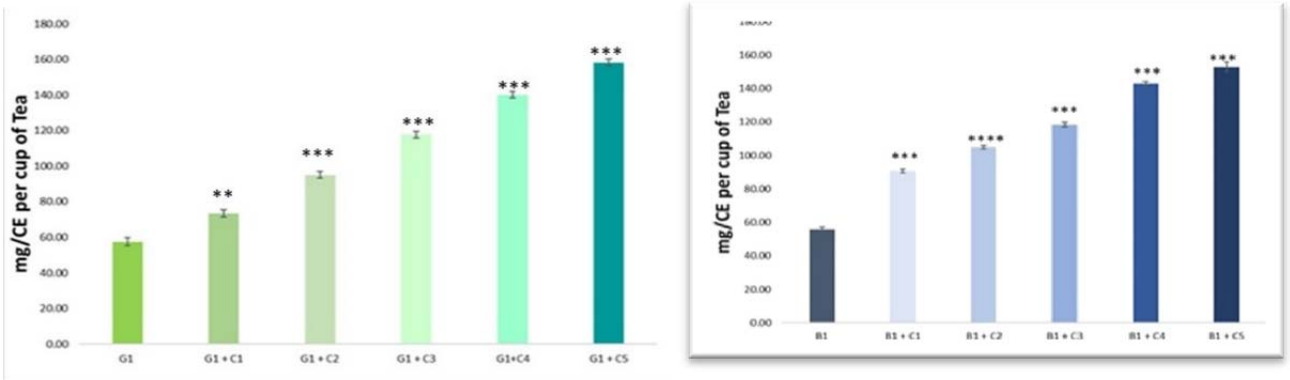

Figure: 2 (a) and (b): Representative graph showing comparison of total flavonoid content (TFC) of original plain green tea (G1) and black tea (B1) as control, infused with varying concentrations of clove (C1; C2; C3; C4; C5). Here ** signifies highly significant.**

Estimation of Total antioxidant content:

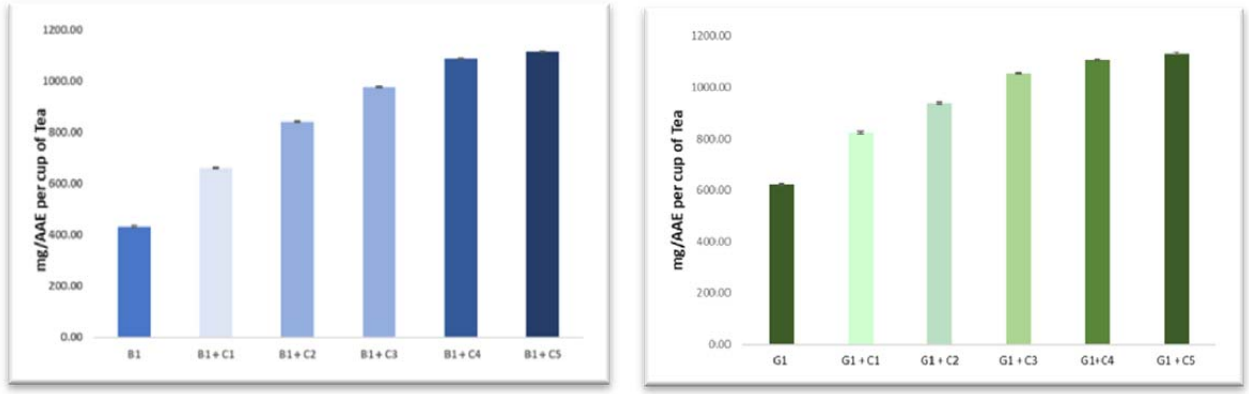

Figure: 3 (a) and (b): Representative graph showing comparison of total antioxidant of original plain green tea (G1) and black tea (B1) as control, infused with varying concentrations of clove (C1; C2; C3; C4; C5). Here ** signifies highly significant.**

In the present study, green tea (GT) and black tea (BT) used as control. The total antioxidant content were determined and compared with green (G1 & G2) and black (B1 & B2) tea infused with varying concentrations of clove respectively. In both the cases like green tea and black tea the order of antioxidant potential follow the same order like TPC and TFC as evident from Fig 3 (a), (b) **and Table 2, 3**.

Table 2: Total Phenolics content (TPC), total flavonoids content (TFC) and antioxidant activity (AA) of black tea infusion along with different concentration of clove.

Sr. No	Sample ID	Composition Tea: Clove	Polyphenol (mg GAE/cup of 100 ml tea) (Average ± SD)	Flavonoid (mg CE/cup of 100 ml tea) (Average ± SD)	Antioxidant (DPPH) (mg AAE/cup of 100 ml tea) (Average ± SD)
1	B1	1:0	351.15 ± 0.58	**55.99 ± 1.23**	432.36 ± 2.99
2	B1 + C1	1:1	586.82 ± 0.22	90.83 ± 0.97	660.31 ± 3.63
3	B1 + C2	1:2	735.19 ± 1.80	104.98 ± 0.93	841.96 ± 2.99
4	B1 + C3	1:3	803.91 ± 2.68	118.36 ± 1.43	975.33 ± 2.37
5	B1 + C4	1:4	890.18 ± 0.96	142.77 ± 1.23	1087.33 ± 2.99
6	B1 + C5	1:5	964.62 ± 2.48	152.88 ± 2.73	1113.44 ± 2.99
7	B2	1:0	256.47 ± 1.72	36.24 ± 1.43	481.44 ± 2.37
8	B2 + C1	1:1	452.95 ± 0.00	66.72 ± 0.47	694.35 ± 2.47
9	B2 + C2	1:2	650.19± 1.45	101.09 ± 1.64	826.13 ± 4.17
10	B2 + C3	1:3	805.18 ± 1.72	115.71 ± 1.23	1002.64 ± 5.18
11	B2 + C4	1:4	933.19 ± 1.91	128.62 ± 1.43	1092.07 ± 3.82
12	B2 + C5	1:5	950.88 ± 1.23	153.97 ± 1.23	1134.02 ± 1.81

Table 3: Total Phenolics content (TPC), total flavonoids content (TFC) and antioxidant activity (AA) of green tea infusion along with different concentration of clove.

Sr. No	Sample ID	Composition Tea: Clove	Polyphenol (mg GAE) (Average ± SD)	Flavonoid (mg CE) (Average ± SD)	Antioxidant (DPPH) (mg AAE) (Average ± SD)
1	**G1**	1:0	298.85 ± 0.96	57.55 ± 2.16	621.93 ± 4.17
2	**G1 + C1**	1:1	543.30 ± 0.96	73.41 ± 2.10	824.94 ± 4.94
3	**G1 + C2**	1:2	645.73 ± 1.01	95.03 ± 1.89	938.92 ± 3.63
4	**G1 + C3**	1:3	788.38 ± 1.54	117.73 ± 1.89	1054.08 ± 3.82
5	**G1+C4**	1:4	840.94 ± 0.79	140.13 ± 1.94	1107.11 ± 3.56
6	**G1 + C5**	1:5	1007.25 ± 1.75	158.17 ± 2.14	1131.65 ± 4.17
7	**G2**	1:0	244.89± 1.32	39.81 ± 1.32	539.21 ± 3.63
8	**G2 + C1**	1:1	474.20 ± 1.17	82.27 ± 2.35	787.74 ± 6.61
9	**G2 + C2**	1:2	558.57 ± 0.96	105.60 ± 0.97	871.64 ± 3.82
10	**G2 + C3**	1:3	162.69 ± 0.58	115.56 ± 1.94	1003.82 ± 2.06
11	**G2 + C4**	1:4	482.85 ± 1.17	132.51 ± 1.40	1110.67 ± 2.37
12	**G2 + C5**	1:5	427.50 ± 0.58	151.33 ± 1.94	1115.82 ± 18.14

Determination of Antimicrobial Property by Kirby Bauer Disk diffusion method

We further evaluated and compared the antimicrobial properties of different clove based herbal tea infusion. The results obtained were as follows.

Table 4: Comparison of Antimicrobial activity of original plain tea samples and the tea samples infused with varying concentrations of clove, against *S.aureus* by measuring the zone of inhibition (in cm)

			ORGANISM - *Staphylococcus aureus*					
			ZONE OF INHIBITION (in cm)					
SAMPLE	CONCENTRATION ((µl)	ORIGINAL TEA	TEA + C1	TEA + C2	TEA + C3	TEA + C4	TEA + C5	CONTROL
G1	**30**	**0.80 ± 0.00**	**0.93 ± 0.06**	**0.97 ± 0.06**	**1.07 ± 0.06**	**1.20 ± 0.00**	**1.40 ± 0.10**	**-**
G2	**30**	**0.83 ± 0.06**	**0.97 ± 0.06**	**1.00 ± 0.10**	**1.07 ± 0.06**	**1.10 ± 0.10**	**1.17 ± 0.06**	**-**
B1	**30**	**0.97 ± 0.06**	**1.03 ± 0.06**	**1.10 ± 0.00**	**1.13 ± 0.06**	**1.17 ± 0.06**	**1.23 ± 0.06**	**-**
B2	**30**	**0.93 ± 0.06**	**1.00 ± 0.00**	**1.03 ± 0.06**	**1.07 ± 0.06**	**1.13 ± 0.06**	**1.20 ± 0.00**	**-**

Table 5: Comparison of Antimicrobial activity of original plain tea samples and the tea samples infused with varying concentrations of clove, against *E.coli* by measuring the zone of inhibition (in cm)

			ORGANISM - *Escherichia coli*					
			ZONE OF INHIBITION (in cm)					
SAMPLE	CONCENTRATION (µl)	ORIGINAL TEA	TEA + C1	TEA + C2	TEA + C3	TEA + C4	TEA + C5	CONTROL
G1	30	0.80 ± 0.01	0.90 ± 0.00	1.00 ± 0.01	1.13 ± 0.06	1.20 ± 0.00	1.20 ± 0.02	-
G2	30	0.63 ± 0.06	0.83 ± 0.06	0.90 ± 0.00	0.97 ± 0.06	1.03 ± 0.06	1.20 ± 0.00	-
B1	30	1.00 ± 0.00	1.07 ± 0.06	1.20 ± 0.00	1.27 ± 0.06	1.33 ± 0.06	1.40 ± 0.01	-
B2	30	0.87 ± 0.06	0.93 ± 0.06	1.00 ± 0.00	1.13 ± 0.06	1.17 ± 0.06	1.27 ± 0.06	-

The sample extracts of both Green tea (G1 & G2) and Black tea (B1 & B2) along with their respective infusions with varying concentrations of clove exhibited antimicrobial activity (measured in cm as the zone of Inhibition) against the known Gram-positive bacteria *S.aureus* and Gram-negative bacteria – *E.coli* (Table 4,5 and Fig:4,5,6 &7). This study further showed that with the increase in the concentration of clove infused with tea, there was an increase in the inhibition of growth of both *S.aureus* and *E.coli*.

Figure: 4(a)

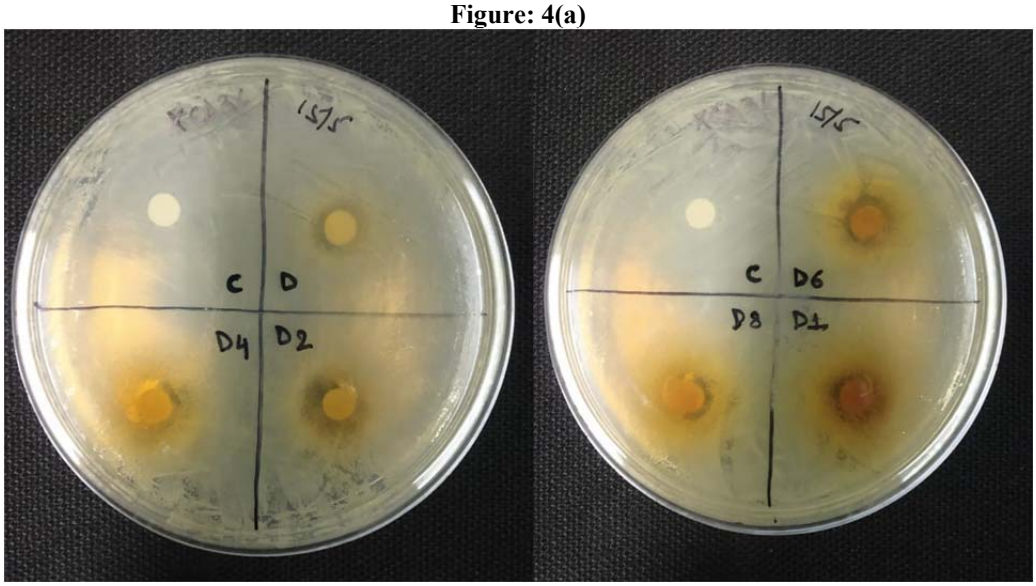

Figure: 4(b)

Figure 4(a): Comparison of the Antimicrobial activity of original plain green tea (G1) and the green tea samples (G1) infused with varying concentrations of clove, against *S.aureus*. (b): Comparison of the Antimicrobial activity of original plain green tea (G2) and the green tea samples (G2) infused with varying concentrations of clove, against *S.aureus*

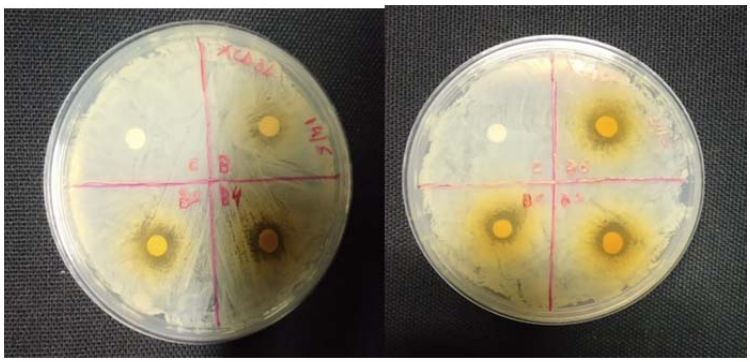

Figure: 5(a)

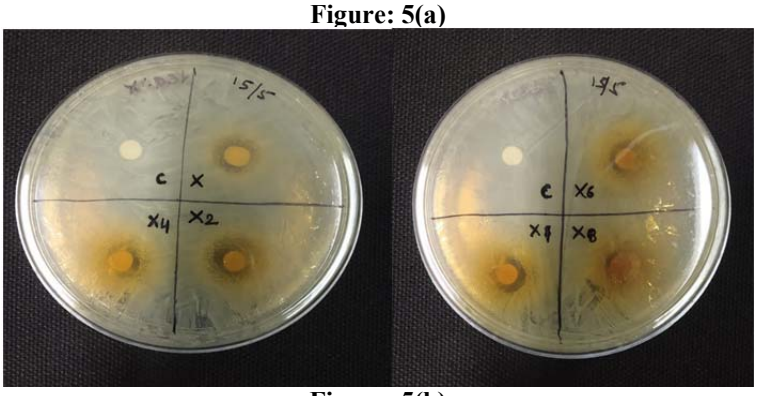

Figure: 5(b)

Figure 5(a): Comparison of the Antimicrobial activity of original plain black tea (B1) and the black tea samples (B1) infused with varying concentrations of clove, against *S.aureus*. (b): Comparison of the Antimicrobial activity of original plain black tea (B2) and the black tea samples (B2) infused with varying concentrations of clove, against *S.aureus.*

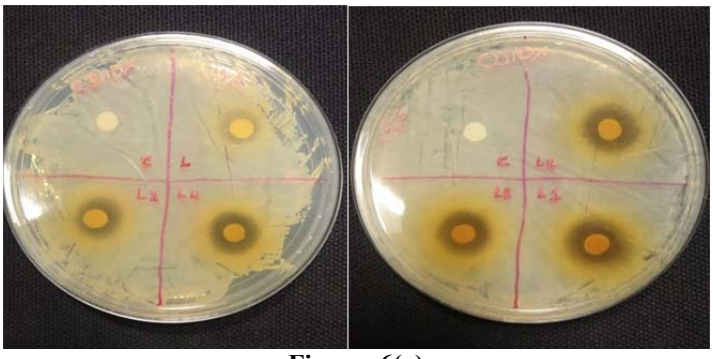

Figure: 6(a)

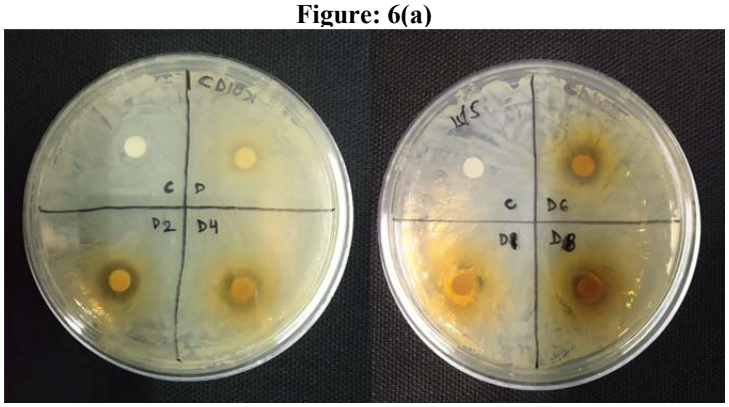

Figure: 6(b)

Figure 6(a): Comparison of the Antimicrobial activity of original plain green tea (G1) and the green tea samples (G1) infused with varying concentrations of clove, against *E.coli*. (b): Comparison of the Antimicrobial activity of original plain green tea (G2) and the green tea samples (G2) infused with varying concentrations of clove ,against *E.coli*.

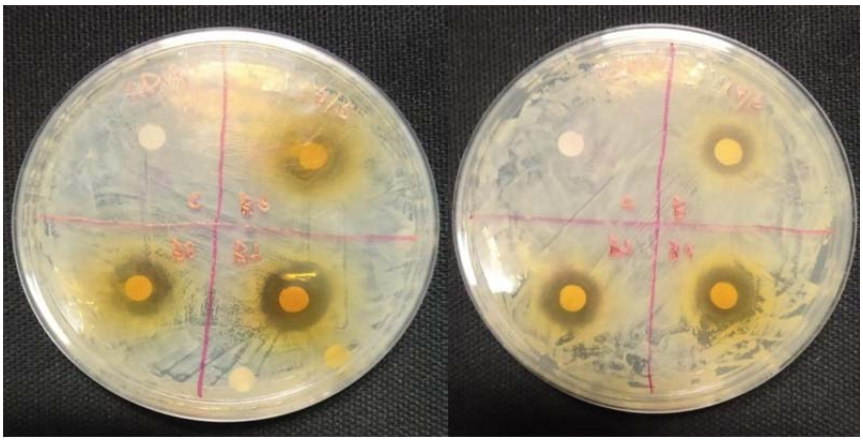

Figure:7(a)

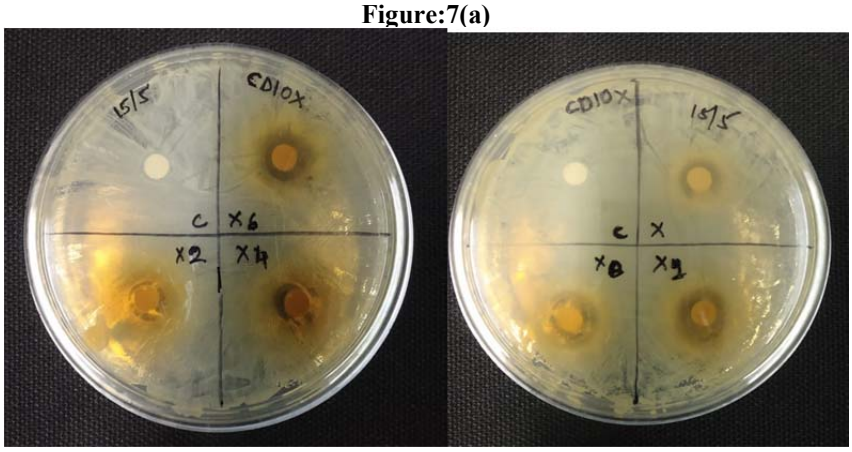

Figure: 7 (b)

Figure 7(a): Comparison of the Antimicrobial activity of original plain black tea (B1) and the black tea samples (B1) infused with varying concentrations of clove, against *E.coli*. (b): Comparison of the Antimicrobial activity of original plain black tea (B2) and the black tea samples (B2) infused with varying concentrations of clove, against *E.coli* .

DISCUSSIONS:

Based on these results, an in-depth study on the clove herbal tea infusions was carried out by brewing varying quantities of clove with fixed quantities of tea in hot boiling water. The results showed that with the increase in the amount of clove in the herbal infusion, the total phenolic content, total flavonoid content, antioxidant potential and its antimicrobial activity against Gram-positive bacteria *S.aureus* and Gram-negative bacteria *E.coli*, increased significantly.

The significant antimicrobial activity exhibited by the clove herbal infusions provides us with an easily available antimicrobial agent, that can fight against bacterial infections.

Moreover, many secondary metabolites that are heat sensitive might get destroyed during the hot decoction preparation time (Chatterjee S 2014). The present study thus designs a way to balance the antioxidant status of hot brewed tea. Therefore, we can say that brewing the tea in hot boiling water along with clove can also fulfil the antioxidant requirement of our body.

CONFLICT OF INTEREST:

The authors have no conflict of interest in the products described in this paper.

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