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Determination of Bioactive Properties of Different Temperature *Camellia sinensis* (Green Tea)

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Abstract Medicinal plants possess an important source of pharmacological effects that acts as new anti-infections, antioxidant and anti-cancer agents. The most important bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides which serve a valuable starting material for drug development. Green tea (*Camellia sinensis)* is widely consumed worldwide. Green tea, the most common type of tea consumed in Asia, contains a large amount of non-oxidized flavonoids, named catechins. There is also evidence that green tea reduces oxidative stress and reverses endothelial dysfunction. The research was conducted to analyze the antimicrobial and antioxidant properties of *Camellia sinensis* (green tea). Keeping in view the importance of flavonoids and other bioactive components this study focused on assessing biological activities using different extracts at different temperature treatments. The disc diffusion method was used to assess the antimicrobial potency of the methanolic and aqueous extracts of *Camellia sinensis*. It was observed that all tested microbial strains were sensitive (>0.82 activity index) to methanolic extract that displayed a higher antimicrobial effect. There was no effect of temperature on the green tea extracts as it followed the same results in case of antimicrobial assay. Similarly, methanolic extract of green tea had high total phenolic (0.33mg/g) and flavonoid contents as compare to aqueous extracts. The present findings show that methanolic extract of *Camellia sinensis* had some high biological activities.

Keywords: *green tea, biological activities, antimicrobial activities, mutagenic activities*

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1. Introduction

Tea is most widely consumed beverage in the world, second in popularity only to water. All black or green tea come from the leaves of single type of ever green plant. Tea provides a powerful antioxidant that probably influence ageing. Tea is also alleged to improve nutrition, prevent tooth decay and reduce obesity, risk of heart disease and cancer may be reducing through tea drinking [32].

Tea beverage is an infusion of variously processed leaves of evergreen shrub. Green tea is widely used every day by billions of people across the world demonstrating its safety. Tea is the most common beverage used in world like water, due to its ability to revive, refresh and relax the body and mind. Tea is generally consumed in the form of black or green tea [24].

Camellia sinensis contains alkaloids and polyphenols, such as flavanols, flavonoids, flavandiols and phenolic acids. Polyphenols commonly called catechins are most common polyphenols found in green tea leaves. Products derived from green tea are mainly extracts of green tea in liquid or powder forms that vary in the proportion of polyphenols and caffeine content [35]. Tea leaves are known for its antimicrobial activity against many microorganisms. Green tea is mainly attributed to its polyphenol contents, particularly flavanols, that are 30% of dry weight of green tea leaves have health promoting effect. Thus, the purpose of this current research is to evaluate the aqueous and methanolic extract of *Camellia sinensis* at differenttemperature (120°C and 170°C) treatment against several biological activities *in vitro* as well as to screen out the cytotoxic effects of green tea.

2. Material and Method

1: Plant

Camellia sinensis (assamica) variety (green tea) were taken from botanical garden of University of Agriculture, Faisalabad. The leaves of green tea were washed with water, and air dried at room temperature, as it contains 21% moisture contents. The samples were ground into a fine powder.

2: Extraction

For aqueous extraction, 10 g of fine powder was soaked in distilled water in sterile conical flask and placed in orbital shaker at 120 rpm for three days. Filtrates were concentrated by using rotary evaporator and stored in refrigerator at 4°C prior to use. Similarly, methanolic extraction was done [1,34].

3: Treatment of green tea (*Camellia sinensis*) extract at different temperature:

Green tea extract was processed at two different temperature treatment (120°C and 170°C) respectively and used them for measuring the different parameters. Green tea along with water boiled at 120°C and 170°C and its temperature was maintained and measured by thermometer.

4: Antimicrobial assay:

Antimicrobial potential of aqueous and methanolic extracts against selected bacterial (Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pasteurella multocida, Agrobacterium tumefacins) and fungal (Aspergillus niger, Aspergillus flavus, Alternaria alternata and Fusarium solani) strains were determined by disc diffusion method [11].

I: Anti-Bacterial assay by disc diffusion method:

Nutrient agar (28.08 g/L) medium was poured in Petri plates and inoculated with the bacterial cultures. Small filter paper discs were impregnated with 30 µL samples of the plant extract. Chloramphenicol (6 mg/mL) and sterilized distilled water were used as positive and negative control respectively. The discs were laid flat on the growth medium and the Petri plates were incubated at 37°C for 24 hours. The extracts having antibacterial activity inhibited the bacterial growth and clear zones were formed. The zones of inhibition were measured in millimeters using zone reader [10].

II: Antifungal assay by disc diffusion method:

Potato Dextrose agar (PDA) (39.06 gm/L) was poured in Petri plates and inoculated with the fungal species. Small filter paper discs were impregnated with 30 µL samples of plant extract, 5 µL Fluconazole (15 mg/250 µL) (as positive control disc) and 30 µL sterilized distilled water (as negative control disc). The plates were incubated at 2°C for 48 hours and the antifungal activity was assessed as discussed above [14].

III: Minimum Inhibitory Concentrations (MIC) of plant extracts:

In 96 well plates (micro dilution plates) poured 100 µL of nutrient broth in all wells. Then added 100 µL samples in first well and dilute it by two-fold dilution method. After that added 20 µL given bacterial culture in all the wells and incubate it for 24 hours at 37°C. The absorbance was measured at 620 nm.

5: Antioxidant activities

I: Total phenolic contents (TPC):

TPC was determined by using Folin-Ciocalteu phenol reagent and absorbance was taken at 765 nm. Whereas gallic acid was used as standard [30].

II: Total flavonoid contents (TFC):

TFC was determined by using $NaNO_2$, NaOH and $AlCl_3$ reagent. Absorbance was measured at 510 nm and catechin equivalent (100-300 ppm) per dry matter used as standard [13].

III: DPPH radical scavenging assay:

DPPH assay was done by using 1-diphenyl-2-picrylyhdrazyl reagent and BHT was used as standard.

$$I\ \% = \left(A_{blank} - A_{sample} / A_{blank}\right) \times 100.$$

Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration [5].

IV: Determination of reducing power:

Reducing power of the given plant sample was determined by using sodium phosphate buffer of pH 6.6 and potassium ferricyanide [K_3Fe (CN) $_6$]. Absorbance was taken at 700 nm. Ascrobic acid was as standard. [26].

6: Cytotoxic analysis

I: Hemolytic assay:

Blood samples were collected in EDTA that was diluted with saline (0.9% NaCl), and centrifuged at 1000xg for 10 min. The erythrocytes separated was diluted in phosphate buffer saline of pH 7.4 and make suspension. Added plant extract and incubated for 5 min at room temperature and add 0.5 mL H_2O_2 that induce oxidative degradation of membrane lipids. Quercetin was take as reference compound. The absorbance was taken at 576 nm by spectrophotometer. Triton 100-X was taken as positive control and saline phosphate buffer were taken as negative control. %age of hemolytic was taken.

II: Anti-thrombolytic assay:

The extract was suspended in DMSO and the suspension was taken vigorously on the vertex mixer. Aqueous preparation of plant extract was added to the eppendorf tube containing the clot to check thrombolytic activity. The streptokinase was used as a positive control for this study.

III: Mutagenicity assay of plant extracts:

The study of mutagenicity and carcinogenicity of green tea extracts were analyzed by Ames test. Mutant *Salmonella typhimurium* (TA98 and TA100) test strains was used to check mutagenicity of aqueous and methanolic extracts of green tea. Ames test was conducted through micro titer plate method. Yellow or turbid wells was scored as positive while purple wells were scored as negative. The extracts of green tea were considered toxic to test strain if all wells in the test plate showed purple coloration. For extracts of green tea to be mutagenic, number of positive well had to be significantly higher than number of positive well in the 'background' (negative control) plate (spontaneous mutation).

7: Inhibition of biofilm:

The quantitative assay for biofilm formation was performed according to the method described by [13]. The glass tubes were filled nutrient broth and inoculated with a microbial strain for overnight. After 48h of incubation at 37°C, the content of each tube was decanted. The tubes were stained with 2% crystal violet for 7 min. Then the tubes were hold with distilled water for 5 min. A positive result was indicated by the presence of an adherent film of stained material on the inner surface of the tube.

8: Statistical analysis

Two-way ANOVA and LSD tests were carried out to assess the significance in the difference of inhibition between the extracts [2].

3. Results and Discussion

3.1. Antimicrobial Activity of Green Tea (*Camellia sinensis*):

The extracts of green tea were screened for antimicrobial activity. Our results indicate that the extracts had a broad spectrum activity against some microbial

species by forming a clear zone of inhibition while against other strains had negligible zone of inhibition and had very poor activity. Average values of zone of inhibition and activity index of aqueous and methanolic extract of green tea were taken is given Table 1.

3.2. Antibacterial Assay

All extracts exhibited comparable degree of activity since standard extraction and assay procedures were performed on a standard amount of dried test material. It was observed (Table 1) that aqueous extract of green tea had more activity index as compared to methanol extract with the exception of *Pasteurella multicoida* (gram negative bacteria). The possible cause of this trend can be that most antimicrobial compounds are more soluble in aqueous solvents and have higher inhibitory strength as compared to methanol extracts. While chloramphenicol was used as positive control (+) and autoclaved water as negative control (-). Difference between results is due to different type of solvent used.

It was observed that methanolic extract of green tea and extract at temperature 120°C and 170°C was more active than aqueous extract. Methanolic extract of green tea have almost same activity index against all the tested microbes (gram positive and gram negative bacterial species). The possible cause of this trend can be that most of the antimicrobial compounds present in green tea are more soluble in methanolic solvent and have more inhibitory effects as compared to other extracts. Similarly, with temperature treatments the antimicrobial compounds would squeezed out as compare to aqueous extracts of green tea.

Bozin *et al*, [8] evaluated the antibacterial activity in the leaf extract of green tea against pathogenic bacteria like *Bacillus subtilis* and *Streptococcus aureus.* The methanolic extract of these leaves were found to possess strong antibacterial activity against a range of pathogenic bacteria revealed by *in vitro* disc diffusion method. Protections of endothelial integrity by elimination of certain risk have proven to be effective in maintaining hemostasis and in slowing the progress of cardiovascular diseases. Indigenous drugs are the natural source of protection against such disorders, which can be used more effectively by the knowledge of their active ingredients as well as by their mechanism of action. Most prominent among them are green tea that played a significant role in reduction of cholesterol level [3].

The minimum inhibitory concentration value which gives a quantitative measure of the resistivity to bacteria is used to further authenticate diameter of inhibition zone results. The general trend of our results coincided with those obtained by Lindequist *et al.* as the MIC values of their methanolic extracts were also higher than aqueous extracts. But this trend was changed as in case of extracts at different temperature treatments 120°C and 170°C. In conclusion, methanol and low temperature treatment extracts were the most efficacious solvents for extracting antibacterial compounds from the green tea. Because at low temperature treatment, active ingredients (catechins and flavonoids) of green tea are in active state because both are secondary metabolites. At high temperature theses active ingredients lost their activity.

3.3. Antifungal Activity

For antifungal activity four fungal strains (*Aspergillus nigher, Aspergillus flavus, Fusarium solani* and *Alternaria alternata*) were used against aqueous and methanolic as well as different temperature treatment (170°C and 120°C) extract of green tea.

From the Figure 1, it is clearly indicated that extract of green tea at temperature 120°C had high activity index against *Aspergillus niger* as compared to other extracts while aqueous extract of green tea had low activity index against *Alternaria alternate*. This was due to the fact that antifungal compounds become less active due to high temperature treatment as it was organic in nature and this was confirmed by the literature of Leta *et al., 2007.*

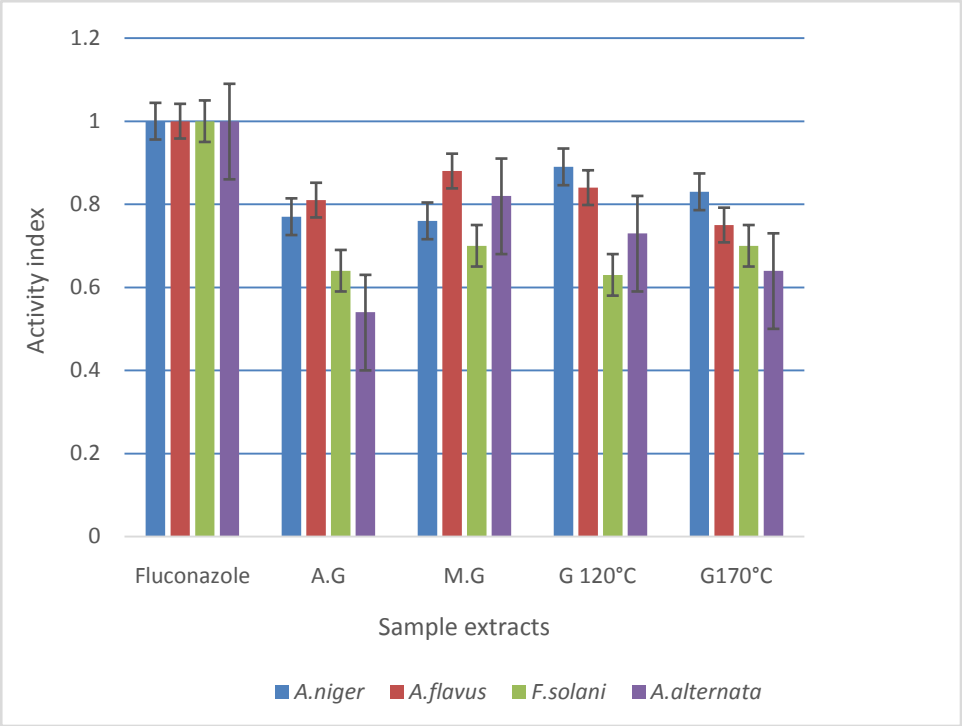
3.4. Antioxidant Activity of Green Tea

The phenolic compounds are one of the largest and most ubiquitous groups of the plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents. Current interest in them stems from their antioxidant, anti-inflammatory, anti-mutagenic and anticarcinogenic activity [4]. Phenols and polyphenols exert their protective effects through diverse mechanism like preventing the formation of carcinogens from precursor substances by acting as blocking agents or suppressing agents [9].

Table 1. Activity index and Minimum inhibitory concentration (MIC) values of different extracts of green tea against Gram positive bacteria and Gram negative bacteria

	Gram positive bacteria				Gram negative bacteria					
	B.subtilis		*S.aureus*		*E.coli*		*P.multicoida*		*A. tumefacians*	
	MIC (µg/mL)	A.I	MIC (µg/mL)	A.I	MIC (µg/mL)	A.I	MIC (µg/mL)	A.I	MIC (µg/mL)	A.I
Aqueous extract of Green tea	1.82±0.51	0.65±0.08	1.57± 0.17	0.60±0.04	1.55±0.40	0.70±0.01	1.61±0.34	0.61±0.09	1.68±0.35	0.52±0.07
Methanolic extract of Green tea	1.83±0.20	0.82±0.09	1.75± 0.46	0.81±0.03	1.67±0.40	0.85±0.04	1.63±0.33	0.82±0.01	1.64±0.40	0.74 ±0.09
Green tea extract at 170°C temperature	2.49±0.48	0.80±0.09	1.27 ± 0.40	0.73±0.07	1.89 ±0.24	0.78±0.32	2.02 ±0.27	0.82±0.09	2.28 ±0.44	0.77±0.10
Green tea extract at 120°C temperature	2.54±0.64	0.74±0.08	1.60 ± 0.65	0.78±0.05	2.12 ±0.36	0.67±0.18	2.18 ±0.36	0.80±0.12	2.30 ±0.57	0.77±0.09
Chloramphenicol	1.86±0.06	1±0.00	1.35 ±0.23	1±0.00	1.86±0.10	1.00±0.00	1.86±0.12	1.00±0.00	1.78±0.06	1.00±0.00

Key: A.I= Activity index, *Bacilllus subtilis=Baciullus subtilis, Staphylococcus aureus= Staphylococcus aureus, Escherichia coli=Escherichia coli, P.multicoida=Pasturellia multicoida, Agrabacterium. tumefacians=Agrobacterium tumefacians.*



Key: *A.niger= Aspergillus niger,A.flavus= Aspergillus flavus,F.solani= Fusarium solani, A.alternata = Alternaria alternata*, temperature, G120°C = Extract of green tea at 120°C temperature, G170°C = Extract of green tea at 170°C temperature, A.G; Aqueous extract of green tea, M.G, methanolic extract of green tea

Figure 1. Antifungal activities of extracts of Green tea

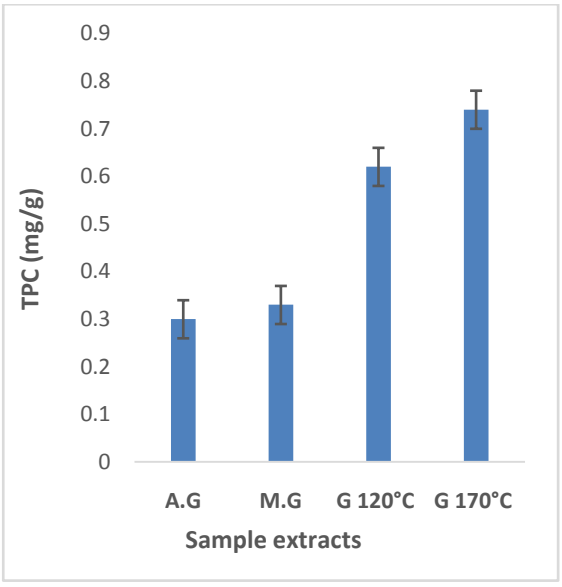


Figure 2. Total phenolic contents of *Camellia sinensis*

3.5. TPC and TFC (mg/g) of Different Extract of Green Tea

From these graphs, it was indicated that extract of green tea at temperature 170°C and 120°C had high phenolic and flavonoid contents as compare to aqueous and methanolic extract of *Camellia sinensis*. The antioxidant activity was correlated with the amount of total phenolics present in the respective extracts in each assay that was comparable with the previously reported work of Siddhuraju [27]. Total phenolic contents were higher in green tea with temperature treatments as compared to aqueous and methanolic extracts. With temperature treatment the phenolic compounds i.e polyphenols and tochopherols become more effective that have antioxidant potential. The TPC is good index of antioxidant compounds in any sample.

Rogerio *et al.*, [23] claimed that flavonoids exert an anti-proliferative action on T cells which can modulate lymphocyte activation and IL-50 production during the Toxpcara infections. Green tea had good total phenolics and flavonoid contents. It exhibited excellent antioxidant activity, as measured by β-carotene bleaching and 1-1-diphenyl-2-picrylhydrazyl (DPPH) assays. It also showed a high superoxide and hydroxyl scavenging activity power and antioxidant activity.

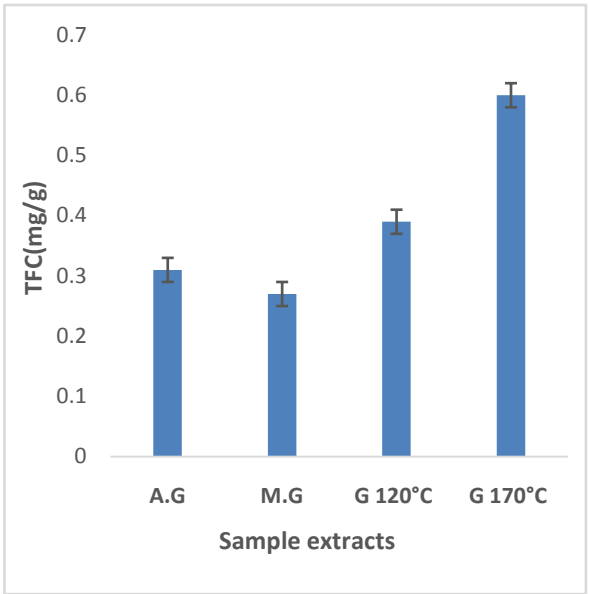


Figure 3. Total flavonoid contents of *Camellia sinensis*

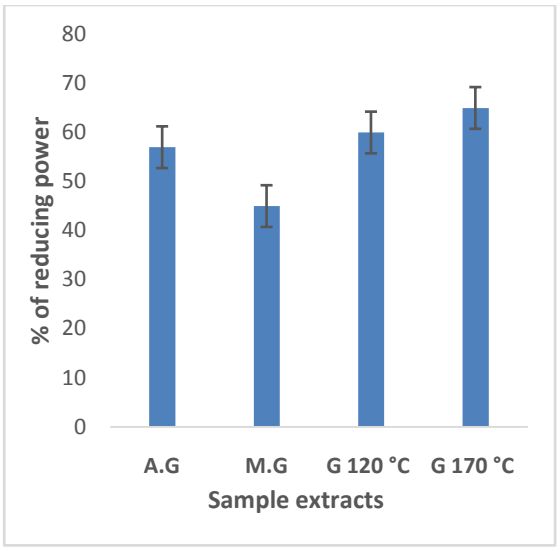


Figure 4. Reducing power of green tea

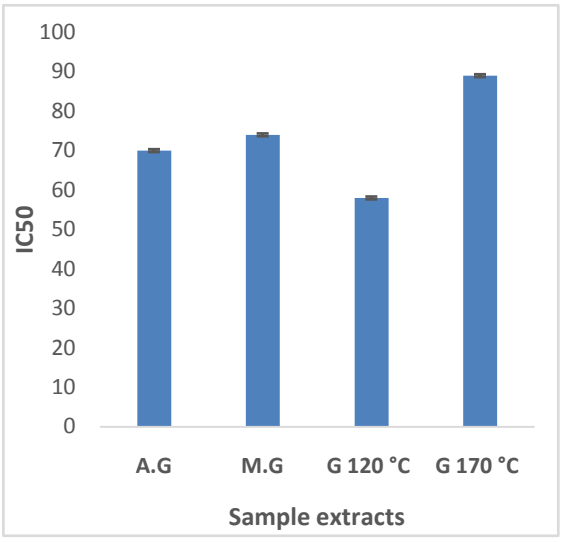


Figure 5. DPPH activity of green tea

3.6. Free Radical Scavenging Activity by DPPH Method Reducing Power of Green Tea

Measurement of reducing potential can reflect some aspects of antioxidant activity of green tea extracts. In this method ferric ions are reduced to ferrous ion with it change in color from yellow to bluish green. The intensity of color depends upon the reducing potential of compounds present in medium. Greater the intensity of color, greater will be the absorption; consequently, greater will be the antioxidant activity [36].

From the Figure 4 and Figure 5 it was indicated that extracts of green tea with temperature treatment 120°C and 170°C have high IC_{50} and reducing power percentage. This was due to the fact antioxidant compounds become more active at high temperature. It was observed that the boiled water extract of green tea had high reducing power that inverse in case of IC50 while the methanol extract obtained decreasing solvent polarity exhibited minimum free radical scavenging activity (62.1%) at 10 mg/mL concentration. The activity of extracts in DPPH assay indicates their hydrogen donating ability as free radicals are known to cause auto- oxidation of the unsaturated lipids in foods.

Mata *et al.*, [19] reported IC_{50} value of green tea as 12 µg/mL of ethanol extract. IC_{50} value was significatly affected by solvent system used. The combined effect of solvents and different plants on radical scavenging activity is significant.

Heavy metal reducing power of extracts/fraction is employed because the antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents; hydrogen donor and singlet oxygen quenchers [30]. The green tea showed the significant reducing potential. These results suggest that green tea basically electro donor reacting with free radicals to convert them into more stable products and to terminate radical chain reactions [7]. Among the extracts/fractions tested\, the water fractions which showed maximum total phenolic contents, exhibited greatest reducing power, including that hydrophilic polyphenolic compounds may be cause of greater reducing power [31]. Similar relation between iron reducing activity and total phenolic content has been reported in literature. However, the correlation may not be always linear [25].

3.7. Antihemolytic Activity

During the drug designing toxicity of active bio molecules is a key factor, and hemolytic activity is a useful starting point in this regard. Hemolytic analysis provides the basic information for the interaction between the active bio molecules and biological entities at cellular level. General indicator of cytotoxicity towards the normal healthy cells is hemolytic analysis of any biological compounds [12]. Plants having active ingredients in the form of saponins (a group of phytochemical) showed hemolytic activity by creating changes in the erythrocyte membrane.

Table 2. Hemolytic activity of green tea

Sample extracts	Hemolytic % age
Aqueous extract of green tea	46.60 ± 0.10^{d}
Methanolic extract of green tea	57.48 ± 0.15^{c}
Extract of green tea at 120°C temperature	73.73 ± 0.26^{a}
Extract of lemon grass at 170°C temperature	67.60 ± 0.20^{b}

Plants having active ingredients in the form of saponins (a group of phytochemical) showed hemolytic activity by creating changes in the erythrocyte membrane. In vitro, for the quantitative measurement of hemolysis, hemolytic assay by spectroscopic method provides effective results. It was observed extract of green tea at temperature 120°C and 170°C have high anti-hemolytic activity as compare to aqueous extract of green tea that was actually compare able with methanolic extract. Hemolytic analysis provides the evaluation of the effect of different concentrations of bio molecules on the human erythrocytes. The ability of the plant extracts to lyse red blood cells at varying degrees depend upon the presence of different types of saponins. Different types of saponins have different hemolytic activities [16]. The effect of anti-hemolytic activity of

green and black teas that were prepared by different ways against peroxide-induced lyses of erythrocytes studied. The strongest hemolytic activity was observed when the boiled extract of green tea was used for the inhibition of hemolysis.

This was not a surprising finding as prolonged tea extraction at high temperatures is needed to obtain maximum yield of substances showing antioxidant activity. The "boiling effect" for the release of antioxidative substances from green tea was not as clear, as the hot water extract of green tea produced stronger inhibition. From the above results it can be seen that the anit hemolytic effect of green tea tea seems to be comparable to that of extract of green tea at different temperature treatments. Actually during boiling process large amount of saponins squeezed out that plays its role as compare to methanolic and aqueous extract of green tea [29].

3.8. Thrombolytic Activity

This study evaluated the thrombolytic potential of plants. Herbal preparations are used since ancient times for the treatment of diseases. Pharmacological investigation had led to discovery of plant derived drugs, which are effective in remedial certain diseases, and renewed the interest in herbal medicines. About 30 % of pharmaceuticals are prepared from plants worldwide [17].

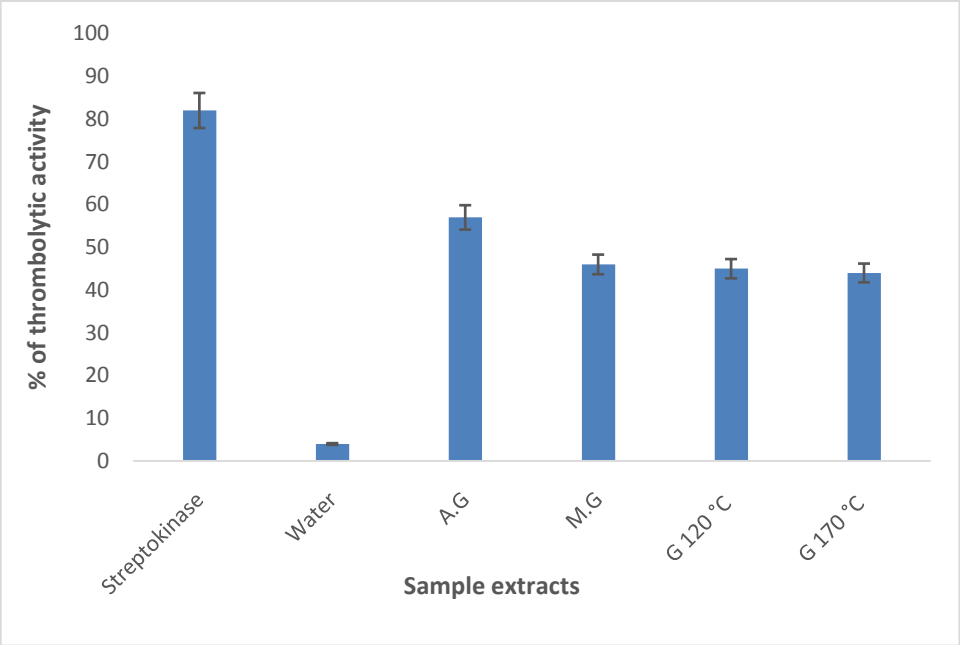


Figure 6. Thrombolytic activity of *Camiellia sinensis* (green tea)

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Camiellia sinensis (Green tea) contains flavonoids and some other related members like aflavins and the arubigins. Other than flavonoids, green tea also possesses a rich amount of cathechins and polyphenols that are expected to have biological activities. Some studies also pointed that as green tea was not fermented during processing preserving the enzymes and olive green color. On the contrary, black tea is fermented before drying. Fermentation can destroy some of the active components of black tea. From our study, in case of aqueous *Camellia sinensis* (green tea) leaf extract maximum 57.56% clot lysis was achieved [28].

3.9. Hydrolysis of Biofilm of Extracts f *Camiellia sinensis*

This assay performed to indicate the hydrolysis of biofilm that indicates potency of active compounds that help to inhibit the biofilm developed by bacteria.

Data from the table indicated that methanolic extract of green tea at temperature have more potency to inhibit the biofilm as compare to other extract of green tea.

Table 3. Anti-biofilm of green tea

Sample extracts	% of inhibition of hydrolysis (INH)
Aqueous extract of green tea	77.56 [c]
Methanolic extract of green tea	90.39 [a]
Extract of green tea at 170°C temperature	78.21 [c]
Extract of green tea at 120°C temperature	82.05 [b]
Positive control (biofilm formation)	92.95 [a]
Negative control (no biofilm formation)	0.00

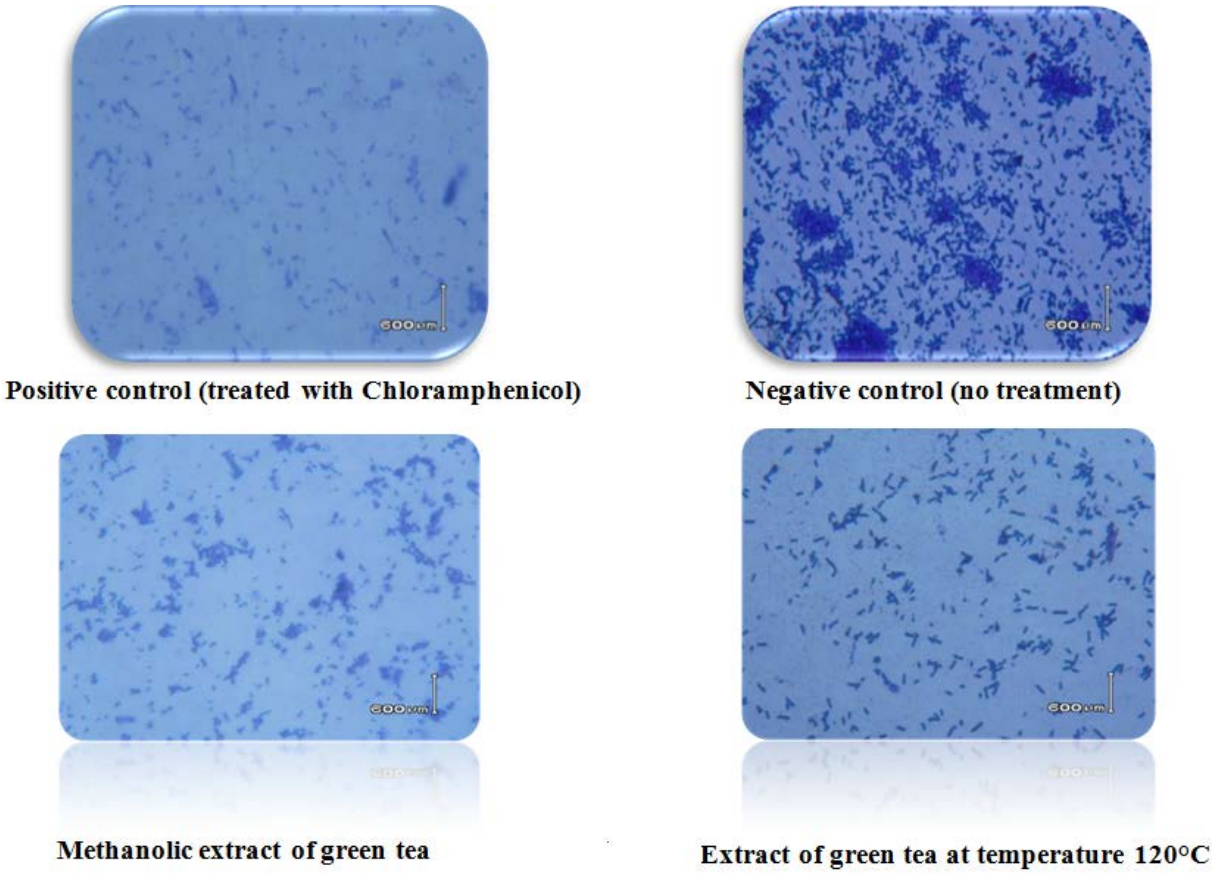

Image 1.

From the microscopic images it was cleared that methanolic extract of green tea inhibit the biofilm of microbes more strongly as compared to other extracts of green tea.

3.10. Mutagencity Assay of Different Extracts of Green Tea

In this study it was found that aqueous and methanolic extracts of lemon grass are more mutagenic towards *Salmonella* strain TA98 and TA100. This was indicated that methanolic and aqueous extract of lemon grass have mutagenic compounds like alkaloids (β carboline). These results suggested that the mutagenicity of TA 98 and TA 100 bacterial strains could be due to the effect of herbal combination and warrant further study on the combined effect of herbal plants in mixed herbal preparation on mutagenicity. This was indicated that Arsenic, Cd and Pb are toxic for human bio-system even at low level of intake. It was concluded that lead may induce to reduce the cognitive development while Cd associated with cardiovascular diseases and affect the kidney function. [20].

Table 4. Mutagenic activity of different extract of green tea using *S. typhimurium* TA 98

Sample extracts	Number of positive well / Number of total wells	Results
Background	20/96	
Standard	92/96	**Mutagenic**
Aqueous extract of green tea	90/96	**Mutagenic**
Methanolic extract of green tea	8/96	**Non mutagenic**
Extract of green tea at 170°C temperature	40/96	**Mutagenic**
Extract of lemon grass 120°C temperature	32/96	**Non mutagenic**

Table 5. Mutagenic activity of different extract of green tea using *S. typhimurium* TA 100

Sample extracts	Number of positive well / Number of total wells	Results
Background	25/96	
Standard	90/96	**Mutagenic**
Aqueous extract of green tea	0/96	**Toxic**
Methanolic extract of green tea	24/96	**Non mutagenic**
Extract of green tea at 170°C temperature	32/96	**Non mutagenic**
Extract of green tea 120°C temperature	32/96	**Non mutagenic**

From the results (Table 4) it was indicated that aqueous extract of green tea showed mutagenic effect as compare to methanolic extract of green tea. Green tea extracts at 170°C temperature was mutagenic as compare to extract of green tea at 120°C.

It was indicated from Table 5 aqueous extract of green tea was toxic for bacterial strain while methanolic extract does not observed significant result. In this study it was found that aqueous and methanolic extracts of green tea was more toxic towards *Salmonella* strain TA98 and TA100. This was indicated that Arsenic, Cd and Pb are toxic for human bio-system even at low level of intake. It was concluded that lead may induce to reduce the cognitive development while Cd associated with cardiovascular diseases and affect the kidney function [20].

4. Conclusion

The results of this study signify the potential of *Camiellia sinensis* as source of medicinal agents. In conclusion, methanolic and extracts at temperature 170°C and 120°C were the most efficacious solvents for extracting antimicrobial compounds. Furthermore thorough studies on the purification of bioactive components, under the proper conditions, can depict the exact potential(s) of the plant as purified components would certainly exhibit more potency with respect to the inhibition of microbes. Furthermore, the activity exhibited by the extracts against microbes may offer scientific justification for medicinal potential of the plant species.

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References

[1] Archana and Jayanthi, A. 2011. Comparative analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens. Journal of Applied Pharmaceutical Science. 01:149-152.

[2] Arora, D. S. *et al.* 2009. Antimicrobial activity of species. Int.J. Antimicribiological agent.12 (7): 257-262.

[3] Ashraf, M. Z., M. E. Hussain and M. Fahim. 2004. Endothelium mediated vaso relaxant response of green tea. Journal of Enthopharmacology. 90: 5-9.

[4] Shahid, M. Z., M. E. Hussain and M. Fahim.2014. Endothelium mediated vaso relaxant response of green tea. Journal of Enthopharmacology. 90: 5-9.

[5] Banejree, S. A., Chakraborty, D. P., k. Suthindhiran and Jayasri, M. A. 2014 Antioxidant and antimicrobial activity of Arucaria cooki and Brassaia actinophyla. Pakistan Journal of Biological Sciences.7 : 715-719.

[6] Baruah, D. B., N. Dash. R. M. Chaudhari and S. S. Kadam. 2006. Plasminogen activators. A comparision. Vascular pharmacology. 44: 1-9.

[7] Bourgou, S., R. Ksouri, A. Bellila, I. Skandrani, H. Falleh and B. Marzouk. 2008. Phenolic composition and biological activities of medicinal plants. C.R. Biologies. 331: 48-55.

[8] Bozin, B., N. Mimica-Duk, N. Simin and G. Anackov. 2006. Chacterization of volatile compositipon of essential oils of some lamiaceae species and the antioxidant and antimicrobial activities of the entire oils. Journal of Agriculture and food chemistry.54: 1822-1828.

[9] Bupesh, G., C. Amutha, S. Nandagopal, A. Ganeskhumar, P. Sureshkumar, K. Saravana Murali.2007. Antibacterial activity of lemon grass from leaf extract. Act Agriculture Solvenica. 89(1): 1854-1941.

[10] Claudine, V., X.W, K.K. Adom and R.H. Liu. 2004. Thermal processing enhances the nutritional value of tomatoes by increasing the antioxidant activity. Journal of Agriculture and food chemistry. 44:497-501.

[11] CLSI (The clinical Laboratory standards institute). 2007. Agar dilution and dics diffusion Susceptibility Testing of campylobacterspp journal of clinical Microbiology. 45(8): 2758-2759.

[12] Da Silva E, Shahgaldian P, Coleman A.W. 2004. Haemolytic properties of some water-soluble para-sulphonato-calix-[n]-arenes. Int J Pharm.2:57-62.

[13] Dewanto, V., X.W, K.K. Adom and R.H. Liu. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing the antioxidant activity. Journal of Agriculture and food chemistry. 44: 497-501.

[14] Dheepa, V., X.W, K.K. Adom and R.H. Liu. 2011. Thermal processing enhances the nutritional value of tomatoes by increasing the antioxidant activity. Journal of Agriculture and food chemistry. 44: 497-501.

[15] Hayam, M. I., M. Ferial, and Abu Salem.2013. Effect of adding Lemongrass and Lime Peel Extracts on Chicken Patties Quality. Journal of Applied Sciences Research. 9(8): 5035-5047.

[16] Inalegwu, B., and Sodipo, O. A. 2013. Phytochemical screening and haemolytic activities of crude and purified saponins of aqueous and methanolic extracts of leaves of Tephrosia vogelii Hook. F. Asian Journal of Plant Science and Research. 3(5): 7-11.

[17] Leta, G. C., P. A.S.Mourao and A. M. F.Tovar. 2002. Human venous and arterial glycosaminoglycans have similar affinity for plasama low density lipo protein. Biochem. Biophys. Acta. 587: 243-253.

[18] Manikndan, A. T., C. Porenca, A. R. Ferreira, M.L.M. Serralherio, J.M.F. Nogueria and M. E. M. Araujo. 2007. Antioxidant and antimicrobial activity of essential oils of fennel extracts. Food Chem. 4: 103-778.

[19] Mata, A. T., C. Porenca, A. R. Ferreira, M.L.M. Serralherio, J.M.F. Nogueria and M. E. M.Araujo. 2007. Antioxidant and antimicrobial activity of essential oils of fennel extracts. Food Chem. 4: 103-778.

[20] Mohd, Y. K., P. Gupta, V. K., Singh, S. Yadav, V. K. Verma. 2011. Cymbopogon Citrates Oil Showing Antimicrobial Activity against Microbes of environmental, clinical and food chain. Asian J. Pharm. (3)2: 67-72.

[21] Nourhan, H. F., Mervat. A. K., Mohamed, A. F., and Fatma, S. E. D. 2008. Influence of aqueous green tea extract on the antimicrobial activity of some antibiotics against Multiresistant clinical isolates. Egyptian Journal of Medical Microbiology. 17: 114-120.

[22] Ratnasooriya, W. D., A. M. T. Amarakoon, T. S. P. Fernamdo, R. A.A.R. Ranatiinga and K.R. W. A, beywickrama. 2007. In vitro anticlotting activity of of Sri Lankan high grown black tea. Sri Lankan Journal of Tea Sciences. 72(1): 23-29.

[23] Rogerio, A. P., A. S. Nunes, D.A. Albuquerque, F. F. Anibal, A. I. Mederiros, E. R. Machado, A.O. Souza, J.C Prado Jr.L.H. Faccioli. 2003. Lafoensia pacari extract inhibit IL-5 production in toxocariasis. Parasite Immunol. 25: 393-400.

[24] Sabu, M. C., Priya, T. T. Ramadasan, K. I. Nishigaki1, C. 2010. Beneficial effects of green tea: A literature review. Chinese Medicine. 5: 13-1.

[25] Saeedeh, A. D. and A. Urooj. 2007. Antioxidant properties of various solvent extracts of green tea leaves. Food Chemistry.102: 1233-1240.

[26] Sebastian tejs. 2008. The Ames test: methodological short review. Environmental biotechnology.4: 7-14.

[27] Siddhuraju, P. and K. Becker. 2003. Antioxidant properties of various extracts of total polyphenolic constitutents from three different agroclimatic origins of lemon grass leaves. Journal of Agriculture and food chemistry. 51: 2144-2155.

[28] Sikandar, K. S., Asma, B. Syeda, S. H. Muhammad Ajmal Shah, Shahana Urooj Kazmi. 2013. Thrombolytic Potential of Aqueous and Methanolic Crude extract of green tea. Journal of pharmaceutical sciences. 23: 12-18.

[29] Simon, M. Ľ. H, M. Greksák, R. Dušinský, and M. Nakano. 2000. Antihemolytic Effect of Rooibos Tea on Red Blood Cells of Japanese Quails. Gen. Physiol. Biophys. 19: 365-371

[30] Sing, G., S. Maurya, M. bP. Lampasonna and C. Catalan. 2006. Chemical constitutents antifungal, and antioxidative potential of lemon grass and its acetone extracts. Food Control. 17 (9): 745-752.

[31] Singh, R., Singh, S.Kumar and S. Arora. 2007. Evaluation of antioxidant potential of ethyl acetate extracts/fractions of Acacia auriculiformis A. Cunn.Food and Chemical Toxicology. 45(7): 1216-1223.

[32] Swati, L. T. S. Pethe, A. C., Tawari and S. P., Rothe. 2012. Antifungal activity of green tea leaves extracts. World Journal of Science and Technology. 2 (6): 23-25.

[33] Tandrima, L. T., A. S. Pethe, A. C., Tawari and S. P., Rothe. 2013. Antifungal activity of green tea leaves extracts. World Journal of Science and Technology. 2 (6): 23-25.

[34] Tariq, A. L., and Reyaz. A. 2012. Phytochemical analysis of Camellia sinensis leaves. International Journal of Drug Development & Research. 4: 7-11.

[35] Tzung, H. T., Tsung, H. T., You-Chia, C. Chi-Wei, L. Po-Jung, T. 2008. In vitro antimicrobial activities against cariogenic streptococci and their antioxidant capacities: A comparative study of green tea versus different herbs. Food Chemistry. 110: 859-864.

[36] Zou, L. M. and S. L. Wei. 2004. Determination of reducing power of essential oils of medicinal plants.J. Biol. Chem. 271: 15081-15025.