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Antibacterial Activity and Phytochemical Profile of Green Tea, Black Tea and Divya Peya Herbal Tea

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ABSTRACT

*Keeping in view the difference in processing techniques of different teas, the present study was planned to evaluate phytoconstituents present and antibacterial activity of various tea (*Camellia sinensis*) extracts against* Staphylococcus, *Streptococcus, Bacillus and multi-drug resistant E. coli. Phytochemical screening to identify major biologically active phytoconstituents in extracts of green tea, black tea and divya peya herbal tea showed the presence of alkaloids, flavonoids, tannins, saponins, reducing sugars and cardiac glycosides in all the tea extracts but steroids were present in black tea only. Ethanolic and methanolic extracts of different tea samples showed significant antibacterial activity against Staphylococcus, Streptococcus, Bacillus and E. coli isolated from soil samples of various locations. Results of zone of inhibition (ZOI) revealed that methanolic extract of green tea, black tea and divya peya herbal tea had highest antibacterial activity (ZOI 19.2, 13.6, 15.8 respectively; AI-1.067±0.006, 0.756±0.005, 0.878±0.010 respectively) again st Bacillus and E. coli showed resistance to aqueous extracts of all tea samples. Results of antibacterial activity and phytochemical screening showed that different processing techniques had no effect on the bioactive components present in tea samples but extraction solvent played important role in extraction of these components as evident from results of antibacterial activity. Overall study indicates that various types of tea can be used as an alternative medicine against the bacterial infections and cariogenic oral flora, opening a promising avenue of clinical applications in the preparation of specific and natural antibacterial and anticariogenic remedies.*

Keywords: *Alkaloids, Black tea, E. coli., Herbal tea, Morphological*

INTRODUCTION

Tea is an aromatic beverage commonly prepared by pouring hot or boiling water over cured leaves of the tea plant *Camellia sinensis* (*C. sinensis)*. After water, tea is the most widely consumed beverage in the world. It has a cooling, slightly bitter, and astringent flavour that many people enjoy. Consumption of tea (especially green) is beneficial to the health and longevity given its antioxidant, flavonoids, polyphenols and catechins content. Tea catechins have known anti-inflammatory and neuroprotective activities, help to regulate food intake[1].

Teas of *C. sinensis* undergo different manufacturing processes. Green tea is produced by steaming (Japan) or panning (China) to prevent catechin oxidation by polyphenol oxidase[2]. Oolong tea is semi-fermented while black tea is fully fermented [3,4,5]. Human studies suggest that green tea may contribute to a reduce the risk of cardiovascular disease and some forms of cancer, as well as promote oral health and other physiological functions such as antihypertensive effect, body weight control, antibacterial and antivirasic activity, bone mineral density increase, antifibrotic properties and neuroprotective powers[6].

Green tea contains mainly flavanols or catechins of epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC).

In black tea, the major polyphenols are thearubigins and theaflavins. The major theaflavins of black tea are theaflavin, theaflavin 3-gallate, theaflavin 3-gallate, and theaflavin 3,3-gallate. Tea polyphenols are also known for their antibacterial activity. In general, antibacterial activity decreases when the extent of tea fermentation is increased, implying stronger activity in green tea than black tea. Green tea catechins, particularly EGCG and ECG, have antibacterial activity. Green tea can prevent tooth decay by inhibiting oral bacteria. The antibacterial activity of black tea has also been reported[7]. Increased enzymatic oxidation of tea results in a decrease in catechin concentration and formation of complexes like theaflavins. Black tea undergoes complete fermentation, oolong tea undergoes partial fermentation, while green and white teas are unfermented[8]. There are number of reports on clinical uses of *Camellia sinensis* in various disorders particularly in cancer and diabetes that have shown promising results[9].

Swami Ram Dev Divya Peya herbal tea is an Ayurvedic drink, free from alcohol, having sweet taste & the best substitute for tea. The main ingredient of Divya Peya is Ashwagandha which strengthens the physical body and relieves it from stress. It is good for general health as it deals with various health issues in the body. It contains cardamom, cinnamon, clove, chandana, black pepper, long pepper, bay leaves, citraka, ashwagandha, shankha-puspi, tulasi, brahmi, dry ginger and saunf. It is boiled for more time than normal tea to boost the effectiveness of the herbs. The bay leaf used treats indigestion, flatulence, gastric, and acidity. The best quality of this drink is that it doesn't harm the unctuousness of milk, and is absolutely free from nicotine and caffeine[10].

Although much work has been done on the phytochemicals present and antibacterial activity of green and black tea but no report was found on Divya peya herbal tea, this prompted us to compare phytocontituents and antibacterial activity of green tea, black tea and Divya peya herbal tea.

MATERIAL AND METHODS

Preparation of Extracts of Tea Samples

Aqueous, ethanolic and methanolic extracts of all the tea samples were prepared by dissolving 50gm tea/50ml solvent (water, ethanol and methanol) in soxhlet apparatus and subjected to extraction. Distillation was carried out for individual tea sample separately and filterate was vaporized to dryness and weighed. The stock solutions of crude extracts were prepared by dissolving the dried extract with respective solvent to obtain the final concentration of 10mg/ml.

Antibacterial Activity of Extracts

To check the antibacterial activity of extracts against bacteria, most prevalent oral bacteria were isolated from soil samples collected from different places by serial dilution method. Isolated bacterial cultures were identified by morphology of colony and gram staining and by standard biochemical tests[11]. Disc diffusion method was used to check the antibacterial activity of tea extracts. Tea extracts were loaded on sterile individual discs and the loaded discs were placed on the surface of Muller Hinton Agar plates (MHA) plates. The extract was allowed to diffuse at least for 5 minutes and then kept for incubation at 37°C for 24-48 hours. After 24-48 hours of incubation plates were observed for appearance of zones of inhibition (ZOI) around the discs[12]. Zone of inhibition or depressed growth of microorganisms was measured and the 'Activity Index' (AI) for each extract was calculated. Activity index (AI) = Inhibition Zone of the sample/ Inhibition Zone of the standard

Minimum Inhibitory Concentration (MIC) of Tea Extracts

MIC is the lowest concentration of an extract or drug that will inhibit the visible growth of a microorganism after overnight incubation. MIC was determined for tea extract showing antibacterial activity against test pathogens in disc diffusion assay. Broth microdilution method was followed for determination of MIC values[13]. Extracts were resuspended in respective solvent to make 10mg/ml final concentration and then was added to broth media of 96-wells of microtiter plates using two fold serial dilutions. Thereafter 100µl inoculum of standard size was added to each well. Bacterial suspensions were used as negative control, while broth containing standard drug was used as positive control. The microtiter plates were incubated at 37±2°C for 24hours.

Each extract was assayed in duplicate and each time two sets of microtiter plates were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the wells of microtiter plate. The MIC values were taken as the lowest concentration of the extract in the well of the microtiter plate that showed no turbidity after incubation. The turbidity of the wells in the microtiter plate was interpreted as visible growth of microorganisms.

Phytochemical Analysis of Extracts

Presence of alkaloids, tannins, saponins, steroids, reducing sugars, cardiac glycosides and flavonoids in all the tea extracts were checked by using modified method by Edeoga *et al*[14].

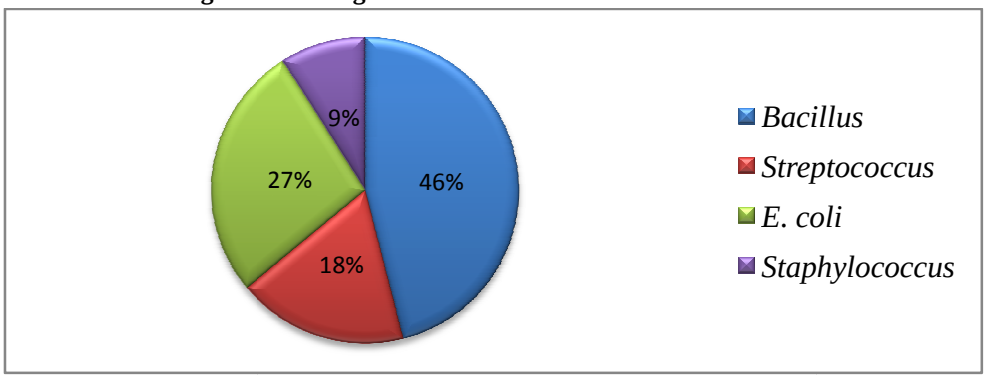
RESULTS AND DISCUSSION

From four soil samples, 11 bacterial isolates were obtained and identified as shown in Fig. 1. *Bacillus* was found to be most prevalent bacteria in soil followed by *E. coli*, *Streptococcus* and *Staphylococcus*.

Antibacterial Activity of Green Tea Extracts

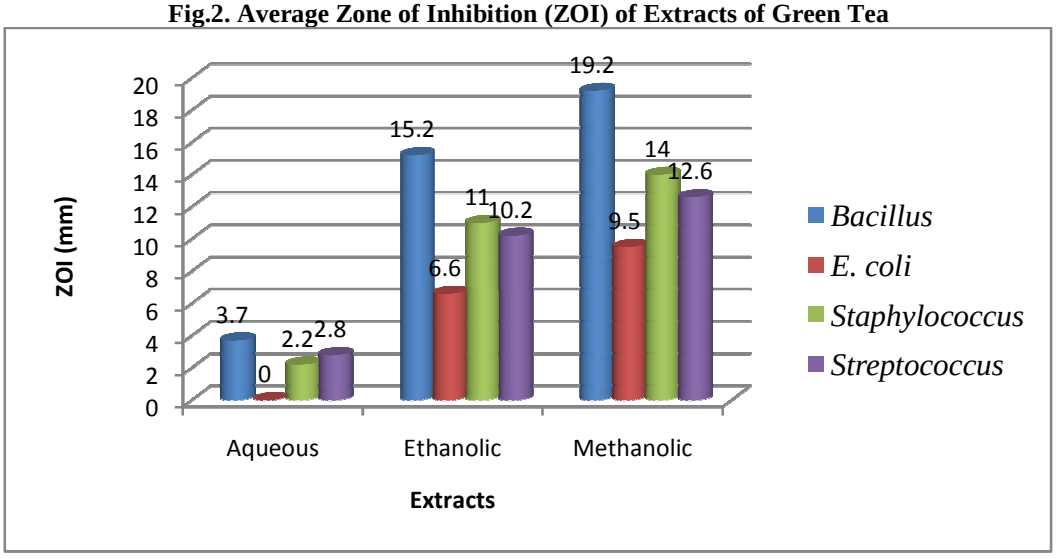
In this investigation total nine extracts were tested and ethanolic as well as methanolic extracts of all the tea samples were highly potent against the tested bacterial strains. Among all the tested bacteria, *Bacillus* was found most susceptible to all the extracts evidenced from zones of inhibition.

Fig. 1: Percentage of Bacterial Strains Isolated From Soil



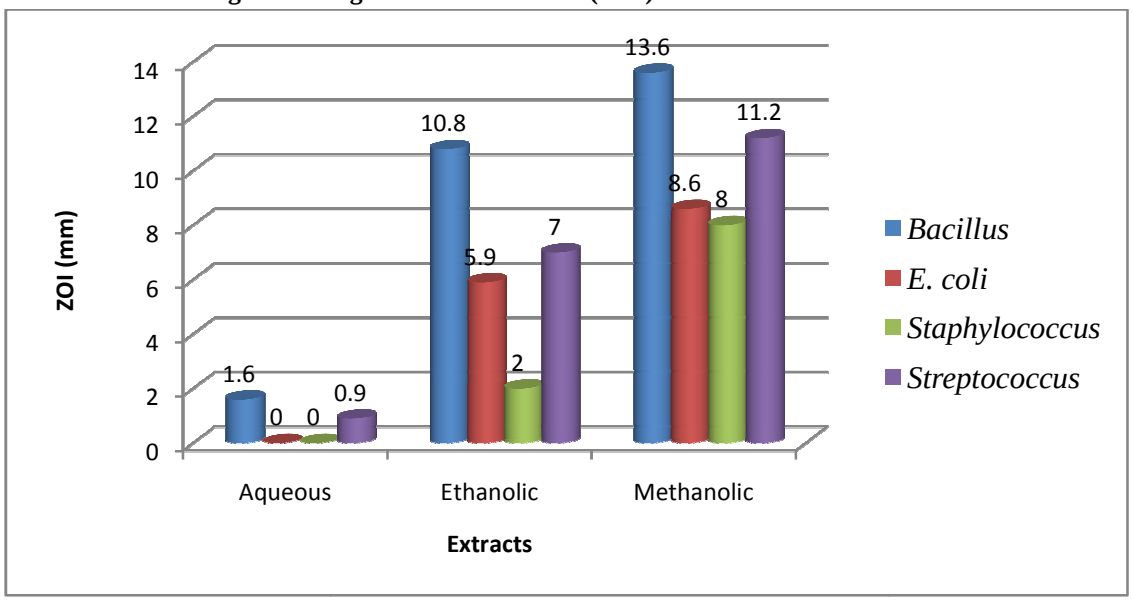
Methanolic and ethanolic extracts of green tea were found to be most effective based on zone of inhibition (ZOI) against *Bacillus* and least against *E. coli* indicating that isolates belonging to *Bacillus* was more susceptible to methanolic (ZOI-19.2mm; AI- 1.067±0.006; MIC 0.042mg/ml) and ethanolic (ZOI 15.2mm, AI 0.844±0.004; MIC 0.084mg/ml) extracts of green tea followed by *Staphylococus, Streptococcus and E. coli. E. coli* isolates were found to be resistant to aqueous extract of green tea as there was no zone of inhibition and very small zones were observed against other bacterial isolates with aqueous extract (Fig. 2, Table 1&2).

Fig.2. Average Zone of Inhibition (ZOI) of Extracts of Green Tea



Bacillus was found to be most susceptible to methanolic (ZOI 13.6mm, AI 0.756±0.005, MIC 0.092mg/ml) and ethanolic (ZOI 10.8mm; AI 0.6±0.057; MIC 0.104mg/ml) extracts of black tea and *Staphylococcus* was least susceptible to both the extracts of black tea (ZOI 8mm & 2mm respectively). *Staphylococcus* and *E. coli* were found to be resistant to aqueous extract of black tea (Fig. 3, Table 1&2).

Fig. 3: Average Zone of Inhibition (ZOI) of Extracts of Black Tea



Like green and black tea extracts, alcoholic extracts of divya peya herbal tea were also more effective against the tested bacterial strains than the aqueous extract and *Bacillus* was most susceptible to all the extracts. *E. coli* was found to be resistant to aqueous extract and other isolated bacterial species were also showing low values of zone of inhibition (ZOI 0-1.5mm) with aqueous extract of herbal tea (Fig. 4, Table 1&2).

Fig. 4: Average Zone of Inhibition (ZOI) of Extracts of Divya Peya Herbal Tea

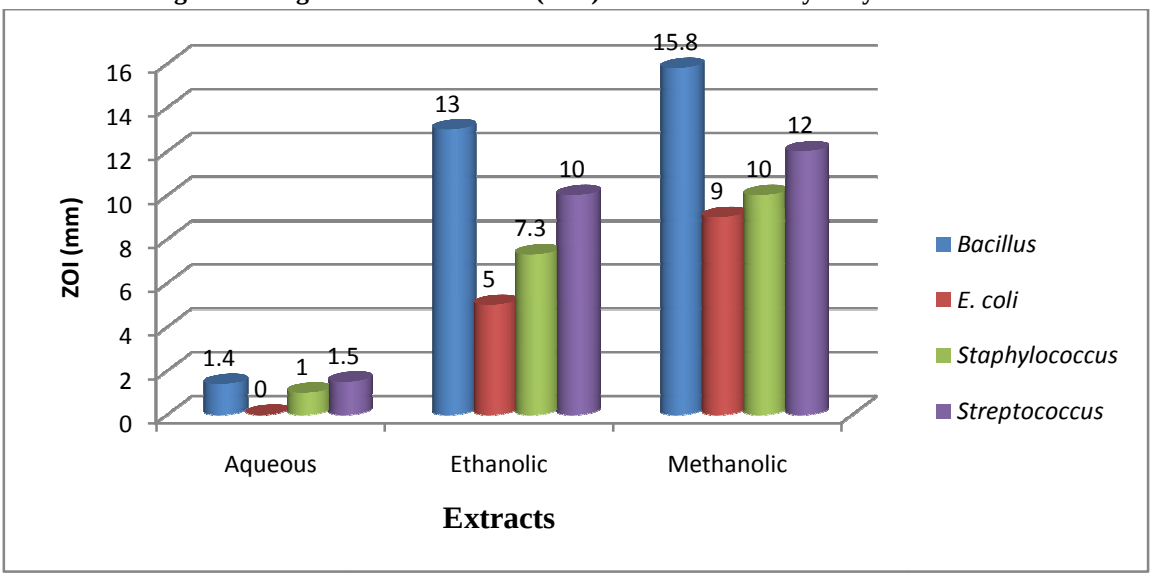


Table 1. Average Zone of Inhibition (ZOI) and Activity Index (AI) of Extracts of Green Tea, Black Tea and Divya Peya Herbal Tea

Tea	Extract	*Bacillus*		*E. coli*		*Staphylococcus*		*Streptococcus*	
		ZOI	AI	ZOI	AI	ZOI	AI	ZOI	AI
Green	Aqueous	3.7	0.205±0.003	-	-	2.2	0.140±0.003	2.8	0.133±0.002
	Ethanolic	15.2	0.844±0.004	6.6	0.253±0.006	11	0.524±0.003	10.2	0.51±0.031
	Methanolic	19.2	1.067±0.006	9.5	0.365±0.005	14	0.667±0.006	12.6	0.630±0.026
Black	Aqueous	1.6	0.089±0.008	-	-	-	-	0.9	0.045±0.005
	Ethanolic	10.8	0.6±0.057	5.9	1.276±0.011	2	0.095±0.002	7	0.35±0.032
	Methanolic	13.6	0.756±0.005	8.6	0.331±0.008	8	0.381±0.011	11.2	0.56±0.032
Divya	Aqueous	1.4	0.078±0.007	-	-	1	0.048±0.014	1.5	0.075±0.008
Peya	Ethanolic	13	0.722±0.006	5	0.192±0.005	7.3	0.348±0.014	10	0.500±0.153
Herbal	Methanolic	15.8	0.878±0.010	9	0.346±0.010	10	0.476±0.013	12	0.600±0.173

ZOI= Zone of inhibition in mm (mean value excluding 6mm diameter of disc)
AI= Activity index (ZOI developed by extract/ ZOI developed by standard)
±= SEM- Standard error of mean; (-) = No activity
Extracts assayed in triplicates
ZOI of standard drug Streptomycin against Bacillus= 18mm, E. coli= 26mm, Staphylococcus= 21mm and Streptococcus= 20mm

Table 2. Minimum Inhibitory Concentration (MIC) Values of Different Tea Extracts against Bacterial Isolates

Bacterial Isolate		*Bacillus*	*E. coli*	*Staphylococcus*	*Streptococcus*
Tea	Extract	MIC	MIC	MIC	MIC
Green	Aqueous	0.328	-	0.374	0.352
	Ethanolic	0.084	0.292	0.092	0.132
	Methanolic	0.042	0.156	0.078	0.104
Black	Aqueous	0.460	-	-	0.620
	Ethanolic	0.104	0.318	0.392	0.240
	Methanolic	0.092	0.182	0.218	0.088
Divya	Aqueous	0.492	-	0.560	0.470
Peya	Ethanolic	0.082	0.382	0.208	0.158
Herbal	Methanolic	0.060	0.208	0.158	0.138

MIC: mg/ml

Phytochemical Analysis of Different Tea Extracts

Aqueous, ethanolic and methanolic tea extracts were checked for the presence of alkaloids, tannins, flavonoids, reducing sugars, saponins, cardiac glycosides and steroids. Qualitative analysis revealed that all the extracts of green tea, divya peya herbal tea and black tea showed the presence of flavonoids, tannins, alkaloids, saponins, reducing sugars and cardiac glycosides but the steroids were present only in black tea. These results of the green tea were found to be similar with the results of previous study of green tea[15]. Phytochemical evaluation of black brand teas revealed the presence of acidic compounds, alkaloids, flavanoids, anthraquinone glycosides, phenolic compounds and tannins, sterols and triterpenoids, saponins, carbohydrates (polysaccharides), proteins and amino acids and absence of resins[16]. The extract showed the presence of phytochemicals namely alkaloids, flavonoids, steroids, gallic tannins and catecholic tannin by changing the colour of the solution to yellow, white, green bluish, blue, green black respectively[17].

Green tea contains flavonoids, tannin, vitamins, fluoride and other mineral salts. Some of antioxidant and antimicrobial agents of green tea could increase the life and efficiency of teeth. Tannins are biosynthetic materials which have a potent antibacterial effect. In recent tooth decay researches, it was mentioned that green and black tea may prevent formation of bacteria in mouth and therefore may reduce construction of plaques on teeth. Also, it was emphasized that the routine consumption of green tea in humans might have reduced the intensity of teeth caries. Using green tea mouthwashes showed a good reduction in bacterial colonies[18].

All the tested bacterial strains were susceptible to ethanolic and methanolic extracts of all the tea samples but aqueous extracts of tea samples were not effective to kill the bacteria.

E.coli. as well as other bacterial strains showed resistance to aqueous extracts to some extent. Methanolic extracts of green tea was more potent against the Gram positive and Gram negative bacteria as compared to the black and divya peya herbal tea extracts. Similar results were found in previous study where aqueous extracts has shown little antibacterial activity against six bacteria isolated. Maximum antibacterial activity was found in methanolic extracts[19]. All the extracts showed highest zones of inhibition against *Bacillus* and these results were found to be similar with earlier results where highest zone of inhibition was observed against *Bacillus subtilis* and *Enterococcus sp.* [19].

It may be suggested that ethanolic ans methanolic extracts of tea can serve as a good source for invention of new therapeutic agents to kill pathogenic bacteria. Ethanolic and methanolic extracts of all the tea samples were inhibitors of the growth of *Streptococcus* and *Staphylococcus*, bacteria responsible for causing dental caries. Results of this study revealed that organic solvents had greater potential for extraction of biologically active compounds and pharmacologically active substances than water and different methodologies used for processing of tea showed no effect on these compounds. It is clear that always organic solvents exhibit the stronger efficiency in extraction of antimicrobial compounds as compared to other methods and a few studies mentioned that organic solvent extracts exhibits the superior antimicrobial activity[20].

CONCLUSIONS

It can be concluded that the extracts of green tea, divya peya herbal tea and black tea were rich source antibacterials and phytoconstituents and all the tea samples were found similar on the basis of phytochemicals and antibacterials. The flavonoids possess anti glycosyl activity and can inhibit adherence of microbes. Tannins can inhibit both glucosyl transferace (GFT) activity and bacterial growth by their strong iron-binding capacity. Alkaloids interfere with the division of cells thus inhibiting their growth. The allopathic antibacterial drugs are said to be costlier and have more side effects. Moreover multiple drug resistant strains are on the rise in this era and thus complicating treatment. On the other hand herbal preparations are comparatively cheaper and have lesser side effects. So, herbal preparations can supplement other systems of medicine for the treatment of diseases caused by bacteria. Further research is required for isolation and identification of main active compounds in the extracts of tea.

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