Pak. J. Bot., 54(2): 667-673, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-2(42)

TOTAL PHENOLIC-FLAVONOID CONTENTS, ANTI-LEISHMANIAL, ANTIMICROBIAL AND ANTIOXIDANT POTENTIALS OF PAKISTANI TEA BRANDS AND TEA PLANT *CAMELLIA SINENSIS*

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Abstract

Tea is generally prepared by steeping the dried young leaves and buds of *Camellia sinensis* in a boiled water. Following water, tea is mostly consuming drink in the world. This research project was designed to evaluate different tea samples for various biological assays. Assays including antibacterial, antifungal, antileishmanial and antioxidant were carried out following standard procedures. Antimicrobial activities were include disc diffusion and micro broth dilution methods, while spectrophotometric method was used for antioxidant and anti-leishmanial assays. Our test samples exhibited considerable antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with MICs of 3.7 µg ml^{-1}. Tapal and Tetly showed considerable inhibitory activities against *R. solani* with 64 ± 3.6 and 76 ± 45% inhibitions respectively at 10 mg ml^{-1} concentrations. Likewise, Lipton and Tapal showed 76 ± 54 and 76 ± 57% inhibitory activity against *A. niger* at 10 mg ml^{-1} concentrations. Tetly was most effective against *L. tropica* with IC_{50} of 293.9 µg/ml. Considerable antioxidant and phenolic, flavonoids contents might be responsible for these pharmacological activities and health benefits associated with the use the selected tea brands.

Key words: *Camellia sinensis*, Bacterial infections, Fungi, Leishmaniasis, Oxidative stress.

Introduction

Tea is the product of dried young leaves of *Camellia sinensis* L. consumed round the globe in plenty amount after the water (Graham, 1992). Studies of Chinese researchers revealed that tea was accidentally discovered during 2700 B. C. by a researcher named Emperor Shen Nong who used hot water for tea extraction (Mair & Hoh, 2012). The first cultivated country of *C. sinensis* is South Asia but at present, it is commonly grown all over Africa, Asia, and several parts of the Middle East (Chopade *et al.*, 2008). Due to its economic importance, tree beverage crop is presently cultivated in over 52 countries in which China and India are top producers (Willson & Clifford, 2012).

Tea is a member of family and genus Theaceae and Camellia respectively. It is an ever favorite tree or bush that reaches to a specific height (wild 10-15 m, cultivated 0.6-1.5 m). Leaves in the young stage have light green in color, alternate, short-stalked, lanceolate coriaceous, serrate margin, pubescent beneath, varying in length (from 5-30 cm and about 4 cm width), while at mature stage leaves are, smooth and leathery and bright green in color. Flowers produced by the tea plant are white fragrant, found lonely or in bunches of two or four having several stamens (Ross, 2005).

C. sinensis has been characterized into three types on the base of the fermentation process during industrialization. The unfermented form is green tea (20%), which is frequently used in Asian countries; fermented teas is black tea (78%), frequently used up in the Western states and a partially fermented oolong tea (2%), produced mostly in southern China (Mukhtar & Ahmad, 2000; Butt & Sultan, 2009). Due to the different processes of manufacturing, teas have their distinguishing colors and flavors. A fermented form of tea is black and red which experience a post-harvested fermentation period before aeration and flowing. Black tea fermentation process is supported by an oxidation process with the help of polyphenol oxidase (enzyme) although red tea is fermentation is done with microbes (Cabrera *et al.*, 2006). Rooibos is another form of tea derives from a South African shrub *(Aspalathus linearis)* having a high amount of antioxidants which is harmless to take by women who are expecting and breastfeeding due to naturally caffeine free nature (Sharangi 2009). Refined conditions for the tea are lands acidity, reasonable temperature and extremely moist atmosphere (Dufresne & Farnworth, 2001). Xanthines, caffeine, tannins, and theobromine, containing polyphenols, flavonoids, vitamin C, and fats are the focal constituents of black tea. A literature review indicated that tea has an extensive series of anti-inflammatory, antioxidant, anticarcinogenic and antibacterial activities in contrast to numerous pathogens (Stagg & Millin, 1975; Diker *et al.*, 1991). Bactericidal potentials of tea preparations are reported by many researchers (Scalbert, 1991). Jarald and Jarald in 2006 reported that tea extract showed anti-tumor, mutation inhibitory, anti-viral, anticancer and lipids lowering potentials (Khursheed *et al.*, 2010).

The emergence of microbial resistance to almost all antibiotics necessitates the discovery and development of novel targets based more effective drugs (Ayaz *et al.*, 2015a; 2016a; 2017). Natural products not only provide a vital alternative source for the discovery of novel

molecules against various diseases but provide a base for the green synthesis of nanoparticles with better efficacy (Chittaranjan *et al*., 2021; Khalil *et al*., 2021). Further, limited efficacy and resistance to anti-leishmanial drugs lead researchers to find more useful alternatives (Nasar *et al.,* 2019; Qasim *et al.,* 2019). In the present study, we evaluated and compared the antimicrobial potentials, anti-parasitic, and antioxidant potentials of commercially available tea brands and field samples.

Materials and Methods

Samples collection and extraction: Three most popular commercially available black tea brands (Lipton, Tapal, and Tetley) were purchased from the local market of Quaid-i-Azam University, Islamabad, while the field samples were collected from National Tea and High Value Crops Research Institute (NTHRI), Shinkiari, Mansehra, Khybert Pakhtunkhwa, Pakistan. The field samples were dried in shade and then grounded to fine powder. Studies of Chinese researchers revealed that tea was accidentally discovered during 2700 B.C. by a researcher named Emperor Shen Nong who used hot water for tea extraction (Mair and Hoh, 2012) and stored in tight jar at room temperature before extraction. For extraction, tea powder (10 g) of all four samples were taken for 2 to 4 h at room temperature in 10 L of 80% Ethanol. Filtration of the extracts were done with the help of muslin cloth followed by Whattman No. 1 filter and kept to dry at room temperature. After drying, different concentration of extracts were dissolved in dimethyl sulfoxide (DMSO) for further analysis (Ayaz *et al.,* 2016b; Zohra *et al.,* 2018).

Phytochemical analysis

Total phenolic content (TFC): TFC of the test samples was assessed according to a previous Folin–Ciocalteu reagent (FCR) method. In a 96 well plate, the volume of 20 µL from ethanolic stock solution (4 mg/mL) from each test sample was shifted in a respective well, with subsequent addition of FCR (90 µL). Incubation of the plate having reaction mixture was done for 5 min followed by addition of 90 µL of sodium carbonate. Gallic acid was used as standard and absorbance of all samples were recorded at 630 nm via microplate reader (Biotek USA, microplate reader Elx 800). Results were expressed as µg gallic acid equivalent per milligram (µg GAE/mg) (Ayaz *et al.,* 2014).

Total flavonoid content (TFC): Previously reported Aluminum trichloride ($AlCl_3$) colorimetric method was used. In a 96 well plate, volume of 20 µL from ethanolic stock solution (4 mg/mL) from our test samples was shifted in a respective well, followed by addition of 1 µL of $AlCl_3$ (10%), 10µL of potassium acetate (1M) and 160 µL of distilled water, kept for 30 min at normal room condition. Thereafter, absorbance was measured via microplate reader at 630 nm. DMSO and quercetin acted as negative and positive control samples. The calculated consequences of triplicate reaction results were expressed as µg quercetin equivalent per milligram (µg QE/mg) extracts (Zeb *et al*., 2016; Zohra *et al*., 2019).

Biological studies

Total antioxidant capacity (TAC): TAC was examined with phosphomolybdenum technique. A composition of the reagent mixture for the classical process is 0.6 M H_2SO_4, 28 mM NaH_2PO_4, 4 mM $(NH_4)6Mo_7O_{24}.4H_2O$. To a total volume of the reaction mixture (200 µL), 80 µL of reagent mixture was added 20µL of test samples. DMSO as a negative and ascorbic acid as positive controls were taken. Interpretations of the triplicate reactions were noted at 630 nm in microplate reader after incubation of the reaction mixture for 90 min keeping temperature at 95°C. Results are expressed as microgram equivalents of ascorbic acid/milligram of the sample (µg AAE/mg) (Mir *et al.,* 2019).

Total reducing power (TRP): A procedure based on Potassium ferric cyanide ($K_3Fe(CN)_6$) was used to study the TRP of four tea samples extracts. To prepare reaction mixture, 50 µL of PBS was added to test sample (the 40 µL) with subsequent incubation for 20 min at 50°C. Thereafter, 50µL trichloroacetic acid (10%) was added. Centrifugation (3000rpm, 10min) of the reaction mixture was accomplished. In a 96-well plate, 33.3 µL of $FeCl_3$ (0.1%) was added to 166.6 µL of supernatant collected from the reaction mixture. DMSO (negative control) and ascorbic acid (positive controls) were used. Absorbance was documented at 630 nm of the triplicate reactions, and the outcomes were then expressed as microgram ascorbic acid equivalents/milligram (AAE/mg) extracts (Imran *et al*., 2014).

DPPH-free radical scavenging potentials: Anti-radicals potentials of the samples were evaluated following our previously reported DPPH anti-radicals method (Khalil *et al.,* 2017; Ovais *et al.,* 2018). Methanolic solution of DPPH was prepared via addition of 2.4 mg (DPPH) to methanol (25mL). Whereas, ascorbic acid and DMSO were used as control solutions. To start reaction, About 200 µL of reaction mixture consisting of 180 µL of DPPH solution and 20 µL of test sample were transferred to a 96 well plate and incubated for 1 h. Absorbance were recorded in triplicate at 515 nm. Percent scavenging was calculated as;

$$\% \text{ Scavenging} = \left\{ \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right\} \times 100$$

Anti-leishmanial studies: Anti-leishmanial study was designed following our previously reported method (Shah *et al.,* 2019a). *Leishmania tropica* KMH23 strain cultures previously grown were adjusted to a cell density of density of 1×10^6 cells/mL (Ayaz *et al.,* 2015b). Subsequently, a MI99 medium (GIBCO) enriched with 10% heat-inactivated fetal bovine serum was used. Aqueous stock solutions (10,000 µg/mL) of the tea samples were prepared and diluted to the required concentration via serial dilutions and added to 96 well plates. Standard drug (Amphotericin B) and distilled water were used as control. Using 96-well, various concentration of tea samples added to selected culture media was were incubated at 24°C for 72 h in a humified incubator with 5% CO_2 and interpretations were taken at

540 nm. After 15 min the persisted promastigotes were counting up with hemocytometer underneath an inverted microscope. Later IC_{50} values were calculated from percent inhibition values using the equation below:

$$\% \text{ Inhibition} = 1 - \left\{ \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right\} \times 100$$

Antimicrobial studies

Bacterial and fungal strains: A sterilized nutrient agar slants (Sigma Aldrich) was used for the growth of used bacterial strains and then sub-cultured onto fresh nutrient agar media. The viability of bacterial strains (Gram-positive; *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus subtilis* while Gram-negative; *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Escherichia coli*) were tested. Cultures of the selected strains were maintained in refrigerator till fresh overnight cultures were prepared from them for anti-microbial tests. Similarly, four fungal strains (*Rhizoctonia solani*, *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*) were grown at 27°C on potato dextrose agar (PDA) (Sigma Aldrich). Fungus spores were collected from cultures on agar plates after 7d as described by (Kamal *et al.*, 2015). Concentration of sporangial suspension was determined via cell counting chamber and diluted to 2×10^6 spores/mL, and stored at -40°C in glycerol (20%). Both bacterial and fungal Strains were collected from the Department of Microbiology at Quaid-i-Azam University Islamabad Pakistan as we reported previously (Sadiq *et al.*, 2016).

Microbial cultures standardization: The microbial cultures were standardized following standard procedure (Aya *et al.*, 2015b).

Disc diffusion method

Antibacterial potentials: Antibacterial potentials of selected pathogenic bacterial strains were performed using disc diffusion assay (Ayaz *et al.*, 2015b). Minimum inhibitory concentration was investigated using broth dilutions assay. A homogeneous microbial lawn (100 µL) was prepared through pasteurized bent glass rod. A 6 mm filter discs loaded with final concentrations of 10 µL and positioned them carefully on the microbial lawns. Gentamycin disc (10 µg) acted as standard drug and DIZ were observed and presented in mm using a Vernier caliper. Further, MICs were assessed via broth dilution technique.

Antifungal assay: An antifungal assay was accomplished by a standard technique (Ayaz *et al.*, 2015b). Tube dilution assay was achieved to find the optimal effective concentration of the ethanolic extract of all tea samples against four fungal strains, applying different concentrations (1, 5 and 10 mg/ mL). PDA media was prepared and transferred to sterilized test tubes. Different extracts of tea samples were then applied to each cooled medium tube and kept in a slanting position. Inoculated strains of the fungus were then applied to the test tubes after slants solidification. PDA slants without plant extract and Amphotericin B served as a Negative control and positive control respectively. After incubation (5 days, at 30°C), for the calculation of linear growth inhibition below equation was used:

$$\% \text{ Inhibition} = 100 - \frac{\text{Growth in sample (cm)}}{\text{Growth in negative control (cm)}} \times 100$$

Statistical analysis: The data of phytochemical, biological, cytotoxic and antimicrobial assays were analyzed using Origin software 8.5. Data were expressed as mean ± SD.

Results and Discussion

Antimicrobial resistance is a major global concern due to emergence of antibiotic resistant superbugs (Ovais *et al.*, 2018; Ovais *et al.*, 2018; Ayaz *et al.*, 2019). Consequently, there is need for the development of novel antimicrobial agents especially from natural sources (Ayaz *et al.*, 2017). The current study was also designed with the aim to appraise the anti-microbial and anti-parasitic potentials of the tea extracts. Tea samples were investigated against different types of bacteria to know antibacterial activities by disc diffusion method. The method used for concentration taken was 3/4 concentration method. The concentration was reduced three times in each step. The different used bacterial strains were *Klebsellia*, *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, *Micrococcus leutus*, *Salmonella typhi* and *Bacillus subtilis*. Table 1 summarizes results of test samples against different strains and their percent inhibition zones.

The results indicated that all four tea samples have antibacterial activities against seven different bacterial different strains. The activity against *Pseudomonas aeruginosa* showed highest while *Klebsellia* spp., indicated lowest percent inhibition. The highest and lowest values of zones were observed in the Tetley sample against *Pseudomonas aeruginosa* and Tapal samples against *Klebsellia spp.* which were 56.4 and 6.4 mm in 100 µg concentration respectively (Table 1). Interestingly the highest zone of inhibition was observed in the field sample against all of these seven bacterial strains. In a study, five different types of tea (Green, black, white, red and rooibos infusion) were used, but the highest scavenging activity was observed in white and green tea (Jafri *et al.*, 2017). Studies have also shown that tea extract act as an inhibitor of food pathogen have studied that green tea is not effective against *E. coli* (Nazer *et al.*, 2005; Almajano *et al.*, 2007).

Minimal inhibitory concentration: The MIC of the tea samples against bacterial strains is listed in the Table 1. According to these results, the MIC of all four samples has shown good results against *P. aeruginosa* and *S. aureus* except the Tetley samples. *S. typhi* also showed good results as compared to *E. coli*, *M. luteus* and *B. subtilis*. Against *Klebsellia spp*. the tea samples were found to be at a higher concentration as compared to the other bacterial strains. Overall antimicrobial activity of tea samples was found to be more against *P. aeruginosa*, *S. aureus*, *S. typhi*, and *Klebsellia* spp. MIC of black and green tea was figured out against *streptococcus aureus* and found elevated MIC of black tea. A previous study on tea (*Camellia sinensis* L.) against *E. coli* and *S. aureus* and the former indicated greater MIC (Taguri *et al.*, 2004).

Table 1. Results of antibacterial study on solvent extracts from various tea brands.

S. No.	Selected microbes	Samples	Concentration used (µg)					
			Gentamicin (+ve control)	100	33.3	11.1	3.7	MIC (µg/ml)
1.	*K. pneumoniae*	Tatly	10	16.2 ± 0.2	4 ± 0.3	1.4 ± 0.3	0.8 ± 0.4	33.3
		Lipton		10.8 ± 0.77	3.5 ± 0.3	1.2 ± 0.25	0.7 ± 0.3	100
		Tapal		6.4 ± 0.4	2.3 ± 0.3	0.6 ± 0.4	0.4 ± 0.3	100
		Field sample		28.4 ± 0.4	9.5 ± 0.4	1.3 ± 0.36	1.4 ± 0.4	100
2.	*Pseudomonas aeruginosa*	Tatly	12.5	56.4 ± 0.4	18.3 ± 0.4	6.2 ± 0.25	2.3 ± 0.25	3.7
		Lipton		52.4 ± 0.4	17.3 ± 0.3	5.5 ± 0.4	1.8 ± 0.3	3.7
		Tapal		50.1 ± 0.4	16.3 ± 0.4	5.2 ± 0.3	1.5 ± 0.3	3.7
		Field sample		38 ± 0.2	12.4 ± 0.4	4.3 ± 0.3	1.2 ± 0.2	3.7
3.	*Escherichia coli*	Tatly	9.2	37 ± 0.5	12.3 ± 0.4	4.4 ± 0.3	1.4 ± 0.4	-ve
		Lipton		29 ± 0.4	10 ± 0.5	3.6 ± 0.4	1.6 ± 0.4	-ve
		Tapal		22 ± 0.4	7.3 ± 0.4	2.4 ± 0.4	0.9 ± 0.3	-ve
		Field sample		11 ± 0.5	3.5 ± 0.3	1.5 ± 0.3	0.7 ± 0.3	-ve
4.	*Staphylococcus aureus*	Tatly	10.5	21 ± 0.5	7.2 ± 0.25	2.1 ± 0.36	1 ± 0.25	3.7
		Lipton		35.3 ± 0.4	12 ± 0.5	3.8 ± 0.3	1.4 ± 0.3	3.7
		Tapal		38.2 ± 0.2	12.4 ± 0.4	4.2 ± 0.3	1.7 ± 0.3	3.7
		Field sample		42 ± 0.2	13.8 ± 0.2	4.4 ± 0.4	1.5 ± 0.5	3.7
5.	*Micrococcus luteus*	Tatly	9.5	28.3 ± 0.2	9.4 ± 0.25	3.3 ± 0.25	1.5 ± 0.3	-ve
		Lipton		33.4 ± 0.4	11 ± 0.4	3.4 ± 0.4	1.5 ± 0.25	-ve
		Tapal		27 ± 0.3	9 ± 0.25	3.3 ± 0.3	1.4 ± 0.4	-ve
		Field sample		15 ± 0.2	5.4 ± 0.3	1.8 ± 0.25	0.7 ± 0.25	-ve
6.	*Salmonella typhi*	Tatly	13.5	26 ± 0.5	9 ± 0.4	2.8 ± 0.3	1.2 ± 0.3	-ve
		Lipton		52 ± 0.2	17 ± 0.3	5.3 ± 0.3	1 ± 0.2	3.7
		Tapal		49 ± 0.2	16.3 ± 0.3	5.3 ± 0.3	2 ± 0.25	-ve
		Field sample		55 ± 0.2	18.3 ± 0.3	6.4 ± 0.3	2.4 ± 0.4	-ve
7.	*Bacillus subtilis*	Tatly	11	34 ± 0.2	11 ± 0.3	3.7 ± 0.25	1.5 ± 0.3	-ve
		Lipton		38 ± 0.4	12.5 ± 0.3	4.2 ± 0.3	1.4 ± 0.4	-ve
		Tapal		37 ± 0.5	12.3 ± 0.3	4.2 ± 0.25	1.5 ± 0.25	-ve
		Field sample		39 ± 0.6	12.5 ± 0.3	4.3 ± 0.4	1.4 ± 0.4	-ve

-ive: Results were not of clinical significance

Table 2. Showing the % inhibition of four fungal strains against tea samples relative to antibiotic Amphotericin B (10 µg) as a positive control using the formula mention in methodology.

S. No.	Organisms	Samples	Percent inhibition		
			1mg/ml	5mg/ml	10mg/ml
1.	*R. solani*	Lipton	18 ± 2.5	26 ± 44	47 ± 33
		Tapal	23 ± 2.5	46 ± 4.5	64 ± 3.6
		Tetly	18 ± 2.5	29 ± 41	76 ± 45
		Field Sample	37 ± 2.5	47 ± 3.5	51 ± 4.5
2.	*A. niger*	Lipton	28 ± 2.5	25 ± 4.5	76 ± 54
		Tapal	28 ± 2.5	46 ± 51	76 ± 57
		Tetly	16 ± 40	46 ± 42	69 ± 44
		Field Sample	16 ± 3.5	30 ± 41	65 ± 57
3.	*A. flavus*	Lipton	28 ± 22	55 ± 53	65 ± 55
		Tapal	26 ± 3.6	26 ± 3.2	46 ± 4.5
		Tetly	17 ± 2.5	25 ± 4.5	45 ± 53
		Field Sample	47 ± 2.5	59 ± 41	72 ± 2.5
4.	*A. fumigatus*	Lipton	18 ± 2.5	44 ± 40	45 ± 51
		Tapal	27 ± 36	35 ± 58	58 ± 70
		Tetly	15 ± 33	46 ± 4.5	63 ± 41
		Field Sample	25 ± 4.5	45 ± 57	46 ± 4.5

Table 3. Results of Antileishmanial potentials of tea samples against *L. tropica*.

S. No.	Samples	% Inhibition					
		700 µg/ml	500 µg/ml	300 µg/ml	100 µg/ml	LC_{50}	LC_{90}
1.	Tetley	100	100	70	0	293.9	536.36
2.	Lipton	100	100	55	0	320.2	530.4
3.	Tapal	100	10	7	0	536.9	800.99
4.	Field Sample	100	6	2	0	551.3	814.4

Antifungal studies: The present study analysis also performed for the antifungal activity of commercial tea brands and field sample. Results showed (Table 2) that all the four tested samples had concentration dependent antifungal activity against the four tested fungal strains. Among the tested samples, both lipton and tetley exhibited 76 ± 54% inhibition each at 10 mg/ml concentration against *F. solani*. Tapal tea extract also exhibited 76 ± 57% inhibitory activity against *A. niger* at 10 mg/ml concentration. Among the other samples, Field Sample (*C. sinensis*) showed 72 ± 2.5% inhibition against *A. flavus* whereas, tetley displayed 63 ± 41% inhibition against *A. fumigatus* at 10 mg/ml concentration respectively.

The lowest percent inhibition (18%) was observed by tetley tea brand against *F. solani* and *A. fumigatus* concentration at 1 mg/ml. In our study, we observed that 10 mg/ml concentration of the samples was the most effective against all four fungal strains.

Anti-leishmanial study: The *In vitro* screening of different medicinal plant extracts for its pharmacological properties is cost-effective and more useful approach (Akbar *et al*., 2020; Chittaranjan *et al*., 2021). Like many other diseases, Leishmaniasis is also an unnoticed neglected tropical disease with several clinical evidences (Yasinzai *et al*., 2013). Its global prevalence is 1-1.5 million annually (Shah *et al*., 2019b), while the parasite has been epidemic in 100 countries (Shah *et al*., 2019c). Early treatment (anti-monial compounds) of Leishmania are not more preferred due to drug resistance and related side effects including nephrotoxicity, abdominal pain, arthralgia, myalgias and cardiovascular complications. Anti-leishmanial potential of the tea samples as compared to other chemical substances showed significant activity as shown in Table 3. The highest LC_{50} observed from the Tetley sample was 293.9 µg ml^{-1} followed by Lipton sample 320.9 µg ml^{-1} and and LC_{90} were 530.4 µg ml^{-1} of Tapal sample followed by 536.3 µg ml^{-1} of Tetley. As leishmania is a tropical disease and is spread via sand fly, so its prevalence is less in developed and cold countries. Subsequently, drug development is also limited due to limited market only in less developed countries. Beside, the emergence of resistant strains is also a major threat to the global health which necessitates the development of more useful therapeutic options for Leishmania. The *In-vivo* effect of aqueous extract of ripen fruits on some biochemical parameters of infected mice with *L. donovani* has also been reported previously with significant results.

DPPH and TAC anti-radicals studies: The potency of antioxidant was checked for 4 different tea extracts (Fig. 1). DPPH free radical scavenging assay was performed for all extracts, and radical scavenging moderated percent average and standard deviation for Tetley, Lipton, Tapal and field sample were 92.4, 92.3, 92.5 and 93 observed respectively at the concentration of 200 µg/mL. The total antioxidant capacity was also observed for all four ethanolic extracts. Hence it was found that, at 200 µg/mL concentration moderated antioxidant average capacity of Tetley was 162.4 mg AAE/mg, Lipton 120.8 mg AAE/mg, Tapal 117 mg AAE/mg and field sample 173.7 ± 0.1mg AAE/mg respectively (Tong *et al*., 2020).

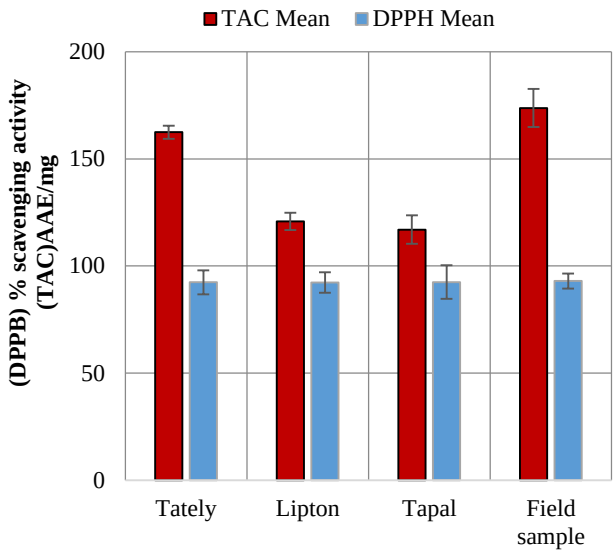

Fig. 1. Results of DPPH and TAC anti-radicals studies.

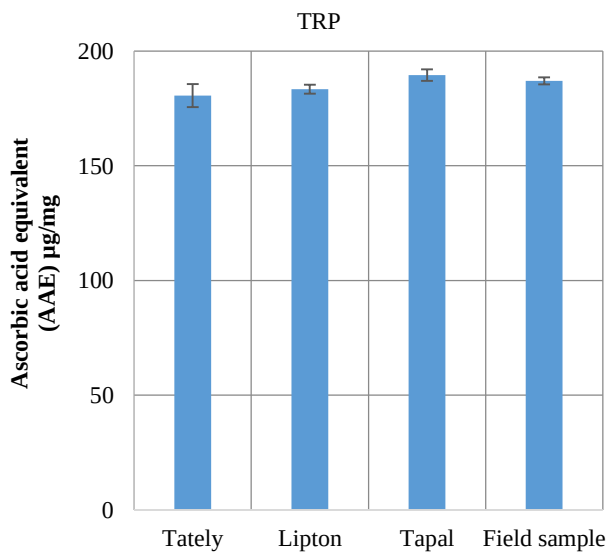

Fig. 2. Total reducing power of various tea samples.

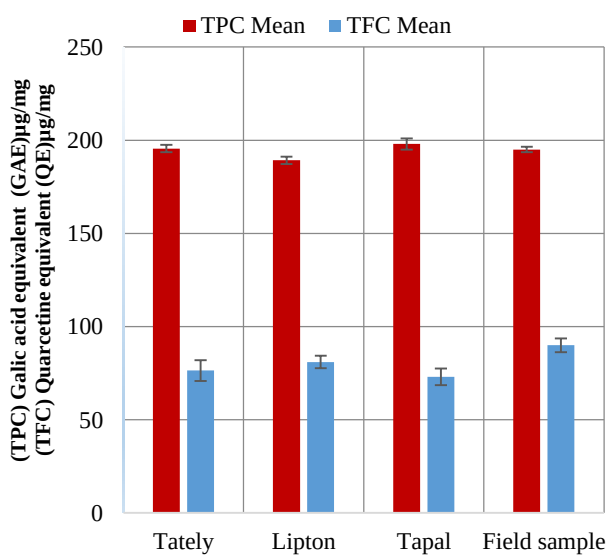

Fig. 3. Results of TPC and TFC of tea samples.

Total reducing power: The antioxidant assay, total reducing power was performed for all four tea extracts (Fig. 2). The average reducing power for Tetley was 180.6 mg AAE/mg, for Lipton it was 183.4 mg AAE/mg, Tapal 189.5 mg AAE/mg and Field sample187 mg AAE/mg respectively at concentration of 200 µg/mL.

Total phenolic-flavonoids contents: All the results evaluation was made to the Gallic acid and Quercetin equivalent. The more significant amount of these extracts characterized the presence of phenolic contents. The phenolic extract average concerning gallic acid was found to be 195.5, 189.2, 198 and 195 (µg gallic acid equivalent/ml of extract) for Tetley, lipton, Tapal and field sample respectively. The standard plot is shown in Figure 3. Estimation of the total flavonoids content of the tea extract was done taking a standard (quercetin) and noted the existence of more flavonoids in the components. The observed average flavonoid content was recorded to be as 76.4, 81, 73 and 90 (µg quercetin equivalents/ml of extract) in tea sample. Hence, it's proved that tea samples have more flavonoid content as compared to phenolic content.

Conclusion

In short, from the current investigational study, it has been deduced that the processed samples of *C. sinensis* (tea) i.e., Tapal and Lipton are possibly more useful than unprocessed field sample and processed Tetley tea due to the presence of more phenolic compounds and high biological potential comparatively. This study can provide a cursory tip to the health care practitioners round the world for the effective of tea in free radicals induced degenerative disorders and microbial infections.

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(Received for publication 25 September 2020)