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COMPARATIVE STUDY OF ANTI-OXIDANT ACTIVITY OF GREEN, BLACK, WHITE AND OOLONG TEA

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ABSTRACT

The present study was investigated to find out anti-oxidant activity of different types of tea by using in vitro DPPH assay and to compare the % inhibition and IC50 values of varieties of tea with standard ascorbic acid. Moreover, phytochemical screening has also been carried out to evaluate the presence and absence of flavonoids, alkaloids, phenols, tannins, proteins and amino acids. The study clearly indicated that the extract of different tea possesses excellent anti-oxidant potential. Ranking of antioxidant properties of tea extracts were found to be green tea > Oolong tea > black tea> white tea. For all four teas, antioxidant property of green tea was significantly higher than extracts of other tea on the basis of % inhibition and IC50 value. The IC50 of green tea, ascorbic acid, oolong tea, black tea and white tea were found to be 2.285, 3.447, 3.607, 4.177 and 7.046 respectively. Hence, it has been proposed that higher polyphenolic content may be the key mechanism for higher antioxidant property compared to other types of tea.

KEYWORDS: Tea, oxidative stress, green tea, reactive oxygen species, ascorbic acid.

INTRODUCTION

Tea is Produced from young leaves of *Camellia sinensis*, it is among one of the most popular beverages.[1] It is consumed worldwide with 0.12 litre/year per capita and is grown in approximately 30 countries. Green tea is prepared by preclude the green leaf poly-phenols oxidation. During the production of black tea, oxidation of most of these substances is promoted. Oolong tea is partially oxidised product. Green tea retains green colour and almost all of original polyphenolic content because no fermentation occurs during the processing.

Oolong tea is semi fermented while black tea is fully fermented.[2] Tea is usually rich in polyphenols, including catechins, thearubigins and theaflavins which are involved in the various health benefits. Tea polyphenols act as antioxidants in vitro by scavenging reactive oxygen species and nitrogen species and chelating redox-active transition metal ions. They may also function indirectly as antioxidants through inhibition of the redox-sensitive transcription factors, nuclear factor-κB and activator protein, inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase (iNOS), lipoxygenases, and xanthine oxidase; and induction of phase II and antioxidant enzymes, such as glutathione S-transferases and superoxide dismutases.[3] It is an evergreen tree or shrub that attains a height of 0.6 - 1.5m when cultivated and 10 - 15 m in the wild.[4]

The leaves are dark green, elliptical or lanceolate, blunt at apex, base is tapering margin shortly serrate. Leaf blade was symmetric, longley oblong or oblong in shape, abaxially pale green and glabrous or pubescent. Young leaves are hairy while young leaves are glabrous.[5] Flowers are white fragrant, 2.5 - 4 cm in diameter, found in solitary or in clusters of two or four. Flowers bear numerous stamens with yellow anther and produce brownish red capsules. Fruit is a flattened, smooth, rounded trigons three celled capsule, seed solitary in each, size of a small nut.[4] Herbal drugs are use of plants for medicinal purposes. About 80% of the population depends on the herbal drugs because of their efficacy, safety and lesser side effects. Herbal drugs have great demand in the developed countries. India has richest medicinal traditional plants in the world. Various advantages of herbal drugs are cheap, easily available, effective with chronic conditions and widespread availability.[6]

The chemical constituents present in tea are catechins, flavonol, aromatic acids, theaflavins, thearubigins, pigments, alkaloids, sugar, amino acid, vitamins, cations, metals, lignans and triterpenoid saponins. It also contains gallic acid caffeine and small amount of theobromine and theophylline depending on the species and horticultural conditions.[7] The polyphenols in tea mainly include the following six groups of compounds: flavanols, anthocyanins, flavonols, hydroxyl-4-flavanols, flavones and phenolic acids. Tea polyphenols which are important and therapeutic are the flavanols of which catechins (flavan-3-ols) are pre-dominant and the major ones are:(-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate(EGCG), (+)-catechin (C), and (+)-gallocatechin (GC).[8] Theaflavins, bisflavanols and theaflavic are responsible for antioxidant properties of tea. These compounds are potent antioxidant in vitro.[9]

Oxidative stress is defined as an imbalance between free radical reactive oxygen species and antioxidant defence mechanism of the body.[10] ROS are highly reactive molecules which can damage cell structures such as nucleic acid, carbohydrates, proteins and alter their functions and lead to cell death.[11] It can cause oxidative DNA damage and lipid peroxidation.[12] Oxidative stress is involved in various pathological conditions such as hypertension, ischemia, cancer, pulmonary fibrosis, diabetes, asthma, acute respiratory distress syndrome and chronic obstructive pulmonary disease.[6] An antioxidant is a molecule that inhibits the oxidation of other molecules and itself gets oxidized. Antioxidants inhibits cellular damage through free radical scavenging property by hydrogen donation. Micronutrients antioxidants are vitamin E, vitamin C and B-carotene.[18] The use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases.

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials with a limited number of antioxidants detected no benefit and even suggested that excess supplementation with certain putative antioxidants may be harmful. As it has been proved that oxidative stress is main cause in the progression of many diseases and polyphenols which have an anti-oxidant activity have shown to prevent neuron degeneration and tumour cell division and other effects such as cardiac protection, hypoglycaemic effect, anti-inflammatory effect and weight loss. Polyphenols are found more prominently in Tea or *Camellia sinensis* herb along with caffeine, amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, fluoride, minerals and trace elements (Cr, Mn, Se, and Zn). Polyphenolsare more concentrated in tea leaves.[19] The main objective of this study was to evaluate the anti-oxidant potential of various types of tea and compared the efficacy with standard ascorbic acid.

MATERIALS AND METHODS

Plant material

The preparation of white tea, green tea, oolong tea and black tea were purchased from Mann tea estate, Dharmshala, Distt. Kangra, Himachal Pradesh.

Method of preparation of extract

Infusions were prepared by adding 200 mL of deionised boiling water to 2 g of tea or herbal tea. The infusions were brewed under cover for 5 min (teas) or 10 min (herbal teas). Then the

mixture was filtered through filter paper and aqueous extract obtained was used for analysis.[13] Extract was then diluted with distilled water to prepare various dilutions of 10, 20, 40, 60, 80 and 100µg/ml.

Phytochemical screening

Aqueous extracts of teas were screened for the presence of various phytoconstituents such as Alkaloids, saponins, glycosides, proteins, amino acids, carbohydrates, terpenoids, tannins, flavonoids, phenolic compounds.[14]

Principle of DPPH Method

DPPH is characterized as stable free radical. The molecule does not dimerize because of delocalisation of spare electron as a whole over the molecule. This delocalisation of electron give rise to the deep violet colour characterized by absorbing band in ethanol solution at about 517nM.[16] Its solution loss characteristic deep blue colour on accepting the hydrogen from a corresponding donor.[15] Change in the optical density of DPPH is monitored in order to evaluate the antioxidant potential through free radical scavenging by the test sample.[16]

Procedure for anti-oxidant activity

The working solutions (10, 20, 40, 60, 80, 100 µg/ml) of the extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml. 1 ml of DPPH solution (0.1 mM in methanol) was mixed with 3 ml of sample extracts and standard solutions separately. The mixture was shaken thoroughly, wrapped with aluminium foil and kept aside for 30 minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of extracts was determined at 517 nm using UV spectrophotometer Higher free radical scavenging activity was indicated by lower absorbance of reaction mixture. Vitamin C was used as reference standard. DPPH (3 ml of 0.1 mM) and methanol (1 ml) was used as blank. DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\% inhibition)} = [(A0–A1/A0) \times 100]$$

Where A0 is the absorbance of the blank and A1 is the absorbance of extract mixed with DPPH. IC50 value (inhibitory concentration at which DPPH radicals where scavenged by 50%) was obtained by interpolation from linear regression analysis.[6,17]

RESULTS

Phytochemical screening

Phytochemical screening of extracts of various types of tea revealed the presence of Alkaloids, saponins, phytosterols, phenols, tannins, flavonoids, proteins and amino acids (Table 1).

Table 1: Phytochemical screening of test compounds and standard.

Tea Types	Alkaloids	Saponins	Phytosterols	Phenols	Tannins	Flavonoids	Proteins and amino acids
Green	+ve	-ve	+ve	+ve	+ve	+ve	+ve
White	+ve	-ve	+ve	+ve	+ve	+ve	+ve
Oolong	+ve	-ve	+ve	+ve	+ve	+ve	+ve
Black	+ve	-ve	+ve	+ve	+ve	+ve	+ve

Antioxidant activity using DPPH free radical scavenging method

Antioxidant activity of various tea extracts were evaluated by DPPH free radical scavenging activity with ascorbic acid as standard. The IC50 was calculated for test compound as well as for standard by linear regression analysis of dose response curve plotting between % inhibition and log concentration as per table-2 and figures 1-6.

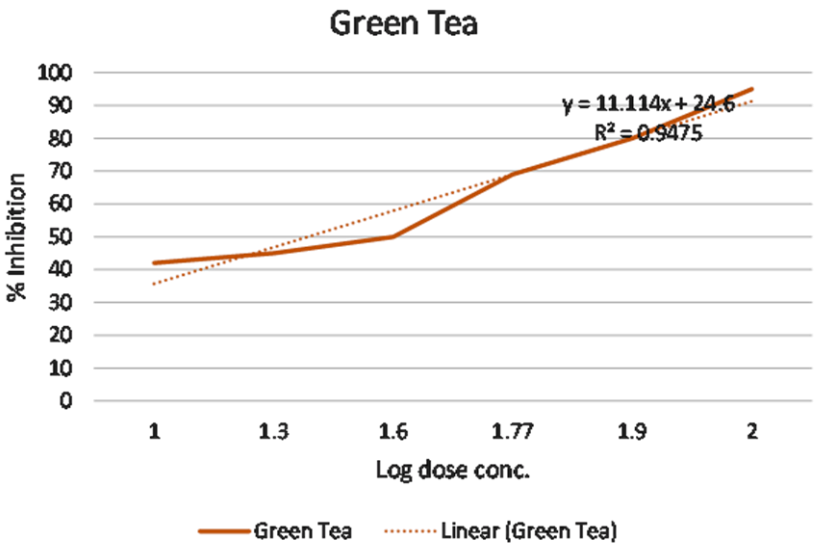

Fig. 1: Effect of green tea as anti-oxidant.

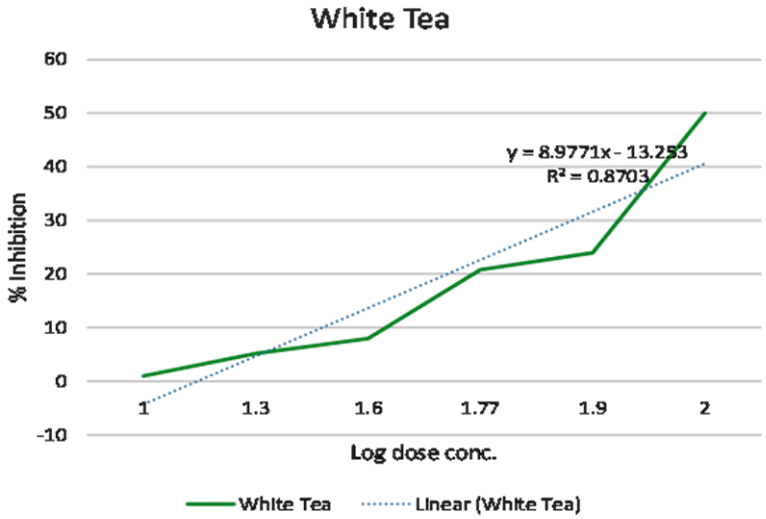

Fig. 2: Effect of white tea as anti-oxidant.

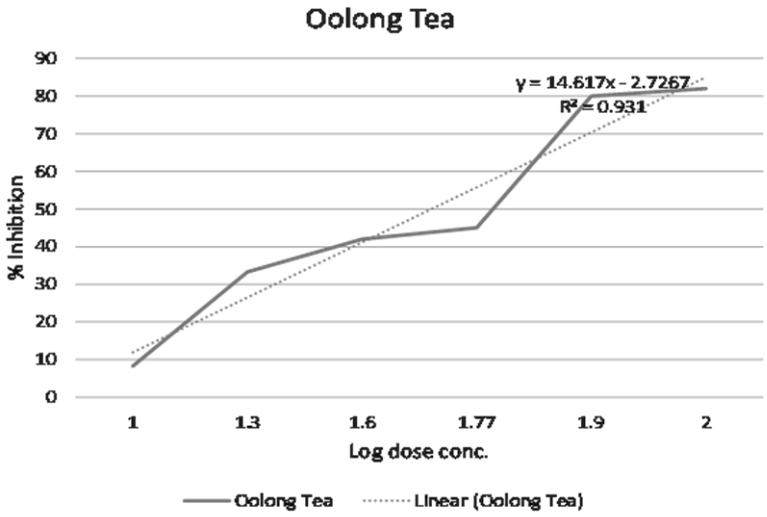

Fig. 3: Effect of oolong tea as anti-oxidant.

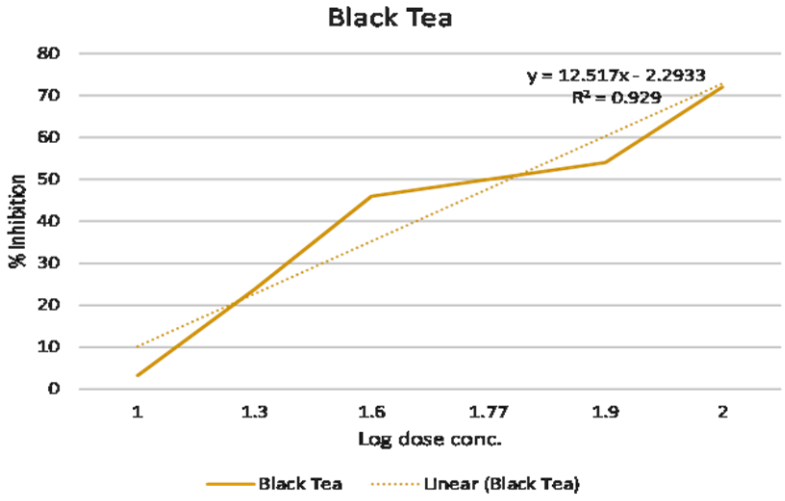

Fig. 4: Effect of black tea as anti-oxidant.

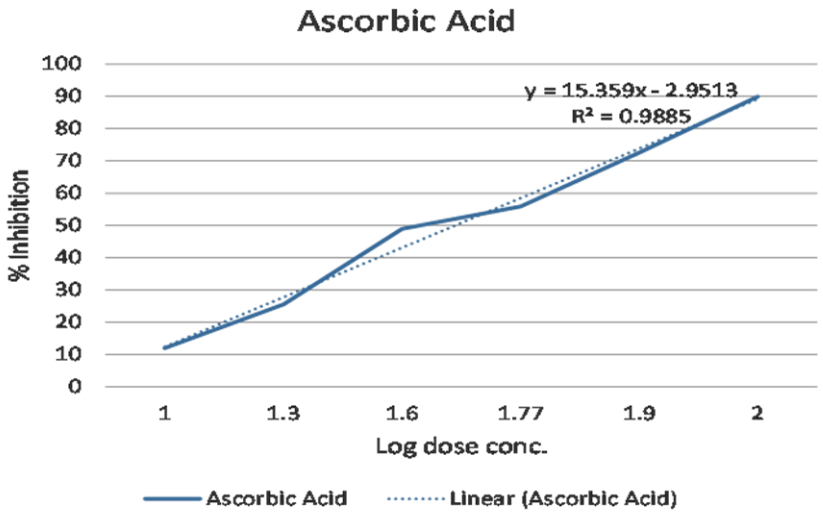

Fig. 5: Effect of ascorbic acid as anti-oxidant.

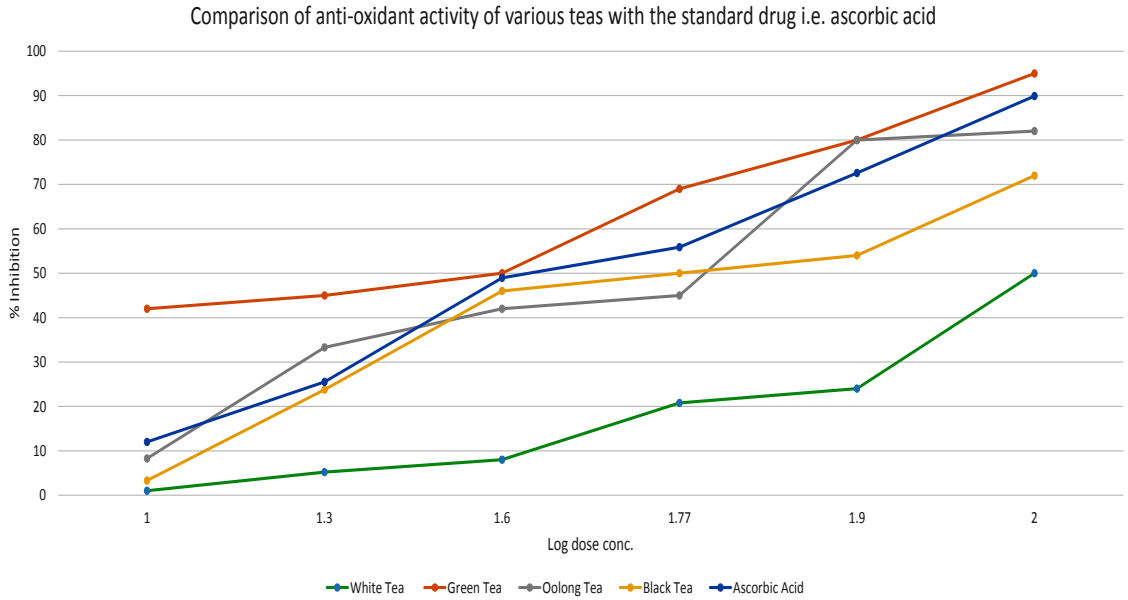

Fig. 6: Comparative anti-oxidant effect of various tea.

Table 2: DPPH free radical scavenging activity of test compounds and standard.

Conc. (µg/mL)	Log Conc.	% Inhibition of different tea samples				% inhibition of ascorbic acid (AA)	IC 50 values of tea samples	IC 50 value of AA
		White (W)	Green (G)	Oolong (O)	Black (B)			
10	1	1	42	8.3	3.3	12	G = 2.285 O = 3.607 B = 4.177 W = 7.046	3.447
20	1.3	5.2	45	33.3	23.8	25.54		
40	1.6	8	50	42	46	48.94		
60	1.77	20.8	69	45	50	55.87		
80	1.9	24	80	80	54	72.59		
100	2	50	95	82	72	89.9		

The result showed that on increasing the concentration of the extract there were gradual increase in the scavenging activity. The descending order of anti-oxidant activity has been mentioned below:

Green tea > Ascorbic Acid > Oolong Tea > Black Tea > White Tea

IC 50 value of green tea, ascorbic acid, oolong tea, black tea and white tea have been found to be 2.285, 3.447, 3.607, 4.177 and 7.046 respectively. IC 50 value of green tea was found to be lower than that of standard ascorbic acid. IC 50 values revealed that Green tea has more scavenging activity than other varieties of tea.

DISCUSSION

In our study green, black, white and oolong tea were used in various concentration of 10, 20, 40, 60, 80, 100µg/mL were used and these showed a potent antioxidant activity by scavenging DPPH radicals. Oxidative stress, a causative agent for each and every diseased conditions in humans. Oxidative stress is either due to increased release of reactive oxygen species (ROS) or decreased anti-oxidant defence of body. Superoxide ions, hydroxyl ion, hydrogen peroxide, nitrous oxide, are ROS causing oxidative stress in the body. Oxidative stress can lead to neuron degeneration, tumour formation, diabetes, aging and others. Moreover, it also is thought that small amount of OS is also necessary for the body, in order to counter act the aging process i.e. repairing of damaged tissue or cell and induction of immune responses in the body. It plays an important role in messenger modulation that regulate cell membrane function.[11,12]

The preliminary phytochemical screening of green, black, white and oolong tea revealed that aqueous extract showed the presence of carbohydrates, flavonoids, alkaloids, terpenoids and phenols. The aqueous extract of *Camellia sinensis* shows anti-oxidant activity at different concentration i.e., 10, 20, 40, 60, 80 and 100µg/mL. In our study, green tea revealed its capacity to scavenge the free radicals by using DPPH method at high concentration followed by oolong, black and white tea, may be due to the presence of alkaloids and polyphenol content i.e., thymol and carvacrol. IC50 value of green tea (2.285) was found to be comparatively lower than the IC50 of ascorbic acid (3.447). Anti-oxidants have great potential in reducing the levels of ROS in the body. They act either by scavenging or by neutralizing the ROS and hence reducing the levels of ROS in the body. Antioxidants are widely used as dietary supplements. However, Clinical trials have also suggested that excess consumption of anti-oxidant is harmful.

CONCLUSION

The study revealed that all the varieties of the tea exhibits anti-oxidant potential. Effect increases as the concentrations of the extracts were increased. Anti-oxidant activity of different teas may be due to presence of polyphenols present in tea extracts. In addition, extract of Green tea has shown maximum scavenging activity and hence antioxidant behavior, which may be due to the presence of higher volume of polyphenolic content compared to other varieties of tea. Therefore, further studies are warranted to elucidate the mechanisms responsible for the anti-oxidant at molecular level.

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CONFLICT OF INTEREST

The Author(s) declare(s) that he has no conflicts of interest to disclose.

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