

EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF TEA LEAVES

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ABSTRACT

The present study was carried out to determine the phytochemical constituents, antioxidant activity and antimicrobial activity in the Different tea samples. Out of eight tea samples tested, leaf extract of Gtee showed highest total antioxidant capacity. Also reducing power activity and hydrogen peroxide activity was found to be highest in Pipple tea extract. The antimicrobial potential of eight tea extracts was screened against eight bacteria *E. coli*, *S. aureus*, *P. vulgaris*, *Pseudomonas*, *Corynebacterium*, *Streptococci*, *Bacillus* and *Klebsiella* sp using well diffusion assay. The tea extracts of Lipton showed significant activity against *Pseudomonas* sp (20mm). Extract of Tetly exhibit highest activity against *P. vulgaris* (17mm) and Organic tulsi showed highest activity against *Klebsiella* (25mm). It can be inferred that the parts of tea having high content of phytochemicals may serve as a good source of nutraceuticals which have potential for use in health care formulations.

KEYWORDS: Phytochemicals, Nutraceuticals, Antioxidant, Phenolics, Healthcare.

INTRODUCTION

Green tea is non-fermented tea. The tea is an infusion of leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols.^[1] Tea is popular not only for its good taste but for its high levels of antioxidants, resulting in its being tested for a variety of health benefits. There are several different types of tea available on the market, including green, black, white, herbal, and oolong. Green tea, made from the leaves of the *Camellia sinensis* plant, is unfermented; the freshly plucked tea-leaf is steam blasted in perforated drums or cooked in iron pans, denaturing its oxidizing enzymes. Herbal tea is not actually tea at all; rather, it consists of an herbal infusion of fresh or dried flowers, leaves, seeds, or roots made by pouring hot water over the plant components and letting them steep.^[2]

The teas obtained from *Camellia sinensis* have been considered as beneficial to human health due to its high contents of phenolic antioxidant compounds. There are two principal varieties of *Camellia sinensis*: var. *sinensis* (Chinese tea) and var. *assamica* (Indian tea) and the levels of phenolics can be significantly affected depending on variety, environmental factors, post-harvest handling and storage conditions, leaf and shoots composition and degree of fermentation.^[3]

Green tea with active chemical ingredients possesses diverse pharmacological properties which are linked to lower incidence of some pathological conditions including oral cancer, dental caries, stroke, cardiovascular diseases and obesity.^[4] Moreover, various reports on antimicrobial, antifungal, antioxidant and cholesterol lowering activities of green tea and its constituents are documented.^[5]

The health-promoting effects of green tea are mainly attributed to its polyphenol contents commonly referred to as catechins. There are four main types of catechins: epigallocatechin-3-gallate (EGCG), epigallocatechin, epicatechin-3-gallate and epicatechin.^[6] The polyphenol contents of green tea have been reported to inhibit varieties of pathogenic bacterial growth such as *Helicobacter pylori*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Salmonella typhi*, *Shigella dysentery*, *Shigella flexneri* and *Vibrio cholera*.^[5] Green tea polyphenols were also found effective against human immunodeficiency virus, hepatitis, and influenza viruses.^[7]

The known in vitro antioxidant properties of catechins and other polyphenolic compounds in tea have led to interest in the potential health benefits of tea consumption.^[8] The evaluation of their efficacy as antioxidants in vivo is more complex.^[9] Numerous epidemiologic studies have addressed the relationships between tea consumption and the incidence of

cardiovascular diseases.^[10] The antioxidant activity of green tea polyphenols and, more recently, the prooxidant effects of these compounds, have been suggested as potential mechanisms for cancer prevention.^[11] The mechanism of action of tea on human health can be characterized not only by potent antioxidant activity (like reduction of LDL oxidation, lipid per oxidation, and DNA oxidation^[12] but also anti-inflammatory (skin disorders, arthritis) and thermogenesis (fat oxidation and energy expenditure) activities as well.^[13]

The objectives of our work were to investigate the scavenging effect of tea extracts on active oxygen and radicals and to find the relationship between antioxidative activity and antimicrobial effect of tea extracts.

MATERIALS AND METHODS

Samples

Different commercial tea bags of green tea were purchased at supermarkets in Mumbai. For each commercial sample, these bags were opened and the contents homogenized. Then, 1 g of the powder was placed back in original bag and resealed.

Extraction of tea samples

One gram of intact tea leaves was extracted with 20ml of methanol for 24h. After extraction, mixture was filtered and obtained extracts were recovered and used for determination of total phenolic compounds, total flavanoids and tannin content, as well as antioxidant activity.

Phytochemical Screening

The methanolic extracts of different plants were used as samples for qualitative phytochemical screening for tannins, alkaloids, saponin, total phenol and flavonoids following the standard procedures of Trease and Evans, 1989.^[14]

Antioxidant activity

Reducing power

The reducing power was based on Fe (III) to Fe (II) transformation in the presence of the solvent fractions.^[15] The Fe (II) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Various concentrations of the sample (2 ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min followed by addition of 2 ml of trichloroacetic acid (100 mg/l). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 2 ml from each of the mixture earlier mentioned was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a higher reducing power.

Total Antioxidant Activity

The total antioxidant capacity of the extract was determined with phosphomolybdenum, using α -tocopherol as standard. An aliquot of 0.2 mL (containing 1.0 mg) of the extract was combined with 2.0 mL of the reagent (0.6 M sulfuric acid, 28.0 mM sodium phosphate and 4.0 mM ammonium molybdate). The blank solution was made by mixing 2.0 mL of the reagent solution with the appropriate volume of the same solvent used to dissolve the sample. The tubes were capped and incubated in water bath at 95 °C for a period of 90 minutes. The sample and blank were left on the shelf for half an hour to cool down to room temperature. The absorbance of the sample was measured against blank solution at 695 nm. A tocopherol graph was plotted by using α -tocopherol as standard and the total antioxidant activity of the plant extract was expressed as μ g - tocopherol equivalent. The equation of the plotted graph is given as: $Y = 5.358x + 0.2427$ where, Y = Absorbance and X = Concentration

Hydrogen peroxide scavenging activity

Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of different fractions was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4) After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank.^[16] The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

Agar well diffusion assay

The antimicrobial activity was measured by Agar well diffusion assay. The plant extract were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Petri plates containing 20 ml Mueller Hinton medium were seeded with the bacterial strains. Each labelled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. Wells were punched and 100 μ l of the methanolic plant extracts were added. The plates were then incubated at 37°C for 24 hours. Erythromycin (0.05%) was used as positive control and analysis was done in triplicates. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The diameter of zone of inhibition can be measured in millimetres.

RESULTS AND DISCUSSION

The phytochemical analysis of the leaf powder and various extracts gave the results as depicted in Table-1.

Table 1: Phytochemical analysis of different tea samples

Tea	Flavanoids mg/ml	Alkaloids gm	Saponin gm	Tanin gm	Total Phenols
Tetly	0.13	0.7	6.93	0.43	0.73
Lipton	0.1	0.66	10.51	1.2	0.95
Organic Tulsi	0.12	0.68	6.34	1.66	0.37
Rose	0.13	1.24	0.14	1.16	0.63
Lemon	0.01	0.94	0.18	0.98	0.58
Gtee	0.02	2.16	0.27	1.13	0.53
Pipple	0.14	1.69	0.18	0.95	0.65
Lemon Grass	0.01	2.2	0.07	1.51	0.57

The curative properties of tea are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, saponins, tanin etc. The successive extracts of leaves of all the eight tea samples have revealed the presence of alkaloids, flavonoids, phenols, saponins, and tannins (Table 1 and Fig: 1,2,3,4,5).

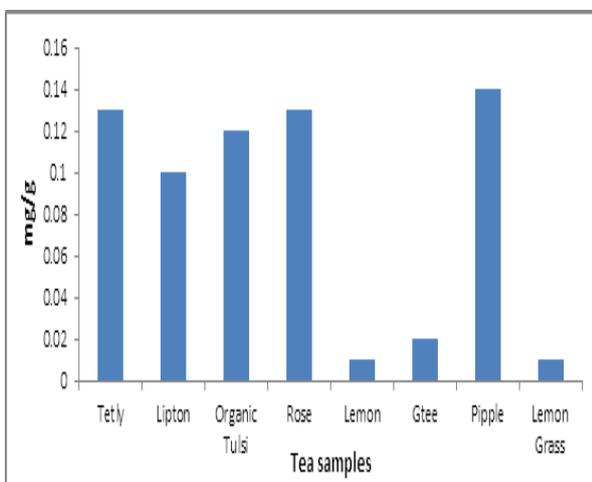


Figure: 1 Flavanoids content of tea samples

The values of flavonoids in different tea samples varied between 0.14 and 0.01 mg/g and were significantly lower in Lemon and Lemon grass tea (0.01 mg/g). The average values of flavonoids of different tea samples showed no significant difference ($p < 0.05$) indicating that these phytochemicals are likely to be responsible for the free radical scavenging activity (Figure 1). Unlike what was observed in the analysis of total phenols, a lower number of brands were significantly different ($p < 0.05$) when considering the same type of tea. Flavonoids are reportedly responsible for the antioxidant activities of plants.^[17] through their scavenging or chelating activity.^[18]

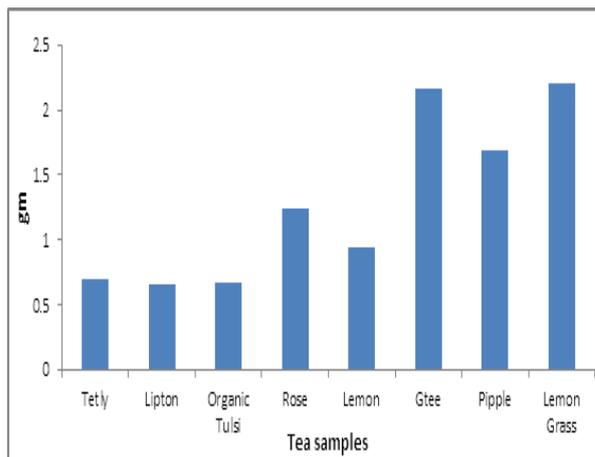


Figure 2: Alkaloids content of tea samples

The values of Alkaloids in different tea samples varied between 2.2g and 0.66g and were significantly lower in Lipton and Organic tulsi tea (Figure 2). The average values of alkaloids of different tea samples showed significant difference indicating that these phytochemicals are likely to be responsible for the free radical scavenging activity.

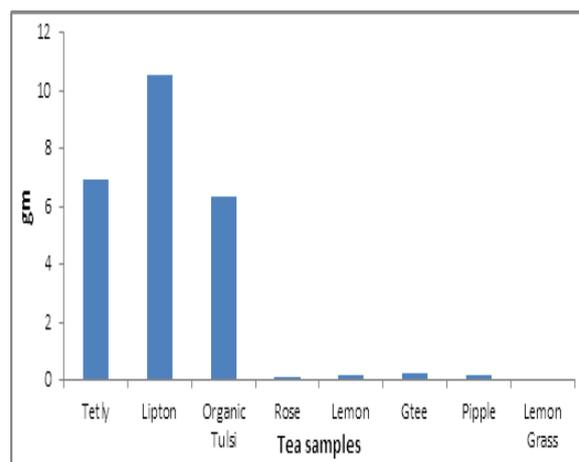


Figure 3: Saponin content of tea samples

Saponin has the property of precipitating and coagulating red blood cells.^[19] Saponin content ranged from 10.51gm to 0.14gm. Lipton tea sample has the highest saponin content of 10.51gm followed by Tetly tea sample with the content of 6.93g. (Figure 3).

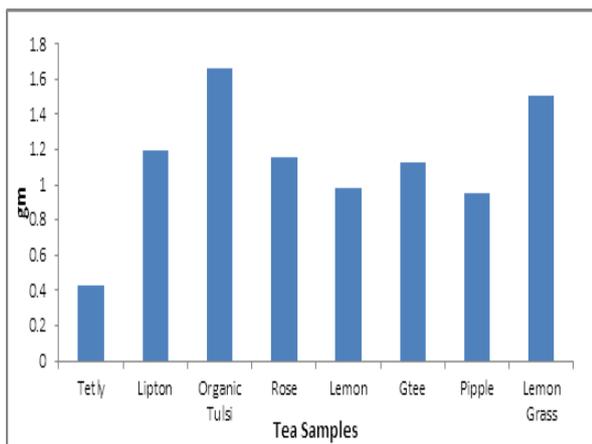


Figure: 4 Tannin content of tea samples

Tannin content of different tea leaves were observed in the range of 1.66 g -0.43g depending on the tea extract. The highest tannin content was found in Organic tulsi and Lemon grass tea extract (Figure 4). Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes.

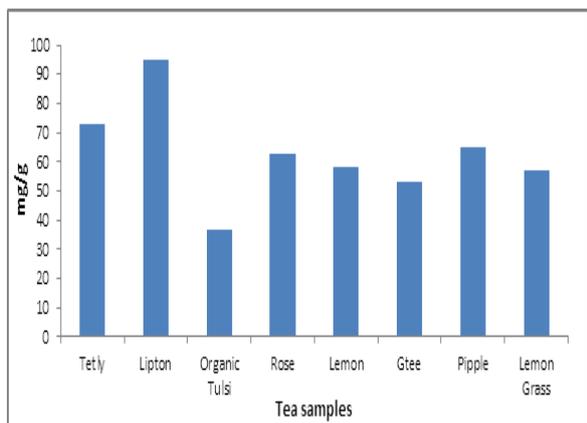


Figure: 5: Total phenolic content of tea samples

Table: 2 Antioxidant activity of different tea samples

Tea	Total Antioxidant activity(µg/mg)	Hydrogen Peroxide activity (mg/ml)	Reducing Power (mg/ml)
Tetly	0.38	0.31	0.84
Lipton	0.7	0.69	1.2
Organic Tulsi	0.06	0.59	1.47
Rose	0.39	0.87	0.62
Lemon	0.41	0.65	0.47
Gtee	1.51	0.85	1.59
Pipple	1.44	0.99	1.7
Lemon Grass	0.4	0.86	0.76

Among the different tea extracts, the greatest antioxidant activity was observed in Gtee tea extracts (1.51), which exhibited inhibition of linoleic acid per-oxidation (Figure 6). Polyphenols are the most abundant group of compounds in tea leaf, and the catechins constitute the major component and seem to be responsible for the antioxidant activity. As shown in Figure 7, the highest reducing powers of green tea were 1.7, 1.59, 1.47 and 1.

Phenolics are well established to show antioxidant activity and contribute to human health. In this study, the total phenolic content was determined using the Folin–Ciocalteu method, with gallic acid as a standard. The content of phenolics was evaluated and expressed in GAE as milligrams per gram of extract (mg GAE/g extract). The total phenolic content of extracts of different tea leaves showed large variations. The lipton tea extracts contained the highest total phenol content (95.00 ± 0.62 mg GAE/g extract), followed by tetly extracts (73.33 ± 2.32 mg GAE/g extract) and pipple tea extracts (65.0 ± 1.12 mg GAE/g extract) (Figure 5). The levels of total phenols obtained in this study are in agreement with those reported in the literature. Sakanaka *et al.* (1989)^[20] found about 50 to 100 mg of polyphenols in a cup (100 mL) of green tea, while Dalluge & Nelson (2000)^[21] reported an average value of 60 mg/g (ranging from 9 to 117 mg/g), depending on the origin of tea.

Antioxidant activity

The analysis of the antioxidant activity of the leaf powder extracts gave the results as depicted in Table-2.

2 at a dose of 1 mg extracts, respectively. The greatest reducing power was observed in pipple tea relative to the other teas (Figure 7). Therefore, tea extracts were electron donors and can react with free radicals to convert them to more stable products and terminate radical chain reaction. Hence, it is supposed that those antioxidant activities may be due to high level of total phenolic compounds.^[22]

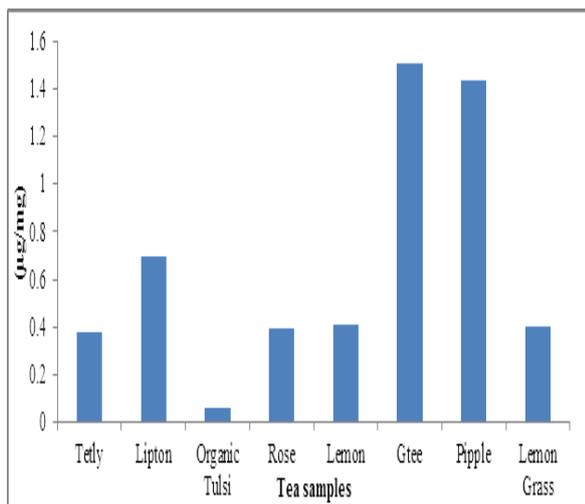


Figure: 6 Total Antioxidant activity of tea samples

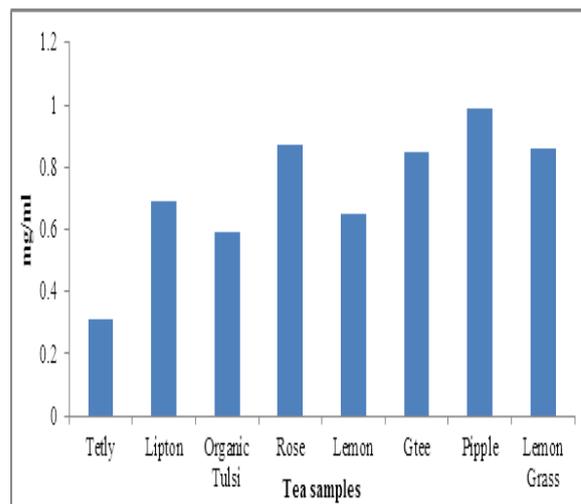


Figure: 8 Scavenging hydrogen peroxide of tea samples

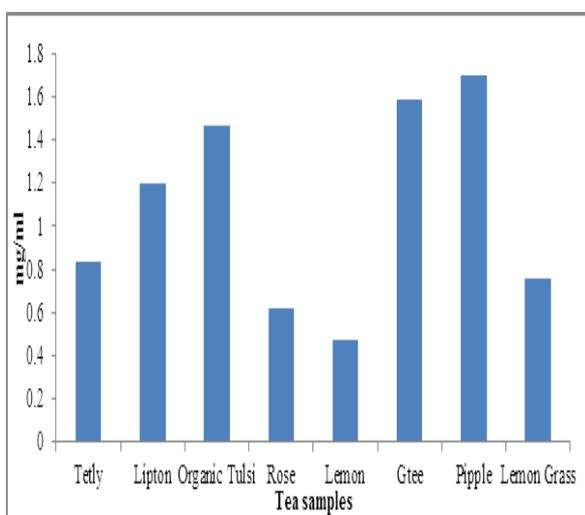


Figure 7: Reducing Power of tea samples

The tea extracts were also capable of scavenging hydrogen peroxide in a manner dependent on concentration (Figure 8). They exhibited scavenging effect on hydrogen peroxide. Among the eight tea extracts, Pipple tea showed the strongest scavenging effect on hydrogen peroxide.

The antioxidant capacity of plant extract is due to the hydrogen donating ability of phenols and flavonoids present in it. Free radicals are constantly generated resulting in extensive damage to tissues and biomolecules leading to various disease conditions. So the medicinal plants are employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress^[23] This could be due to the high flavonoid contents of leaf extract. It has been recognized that flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of most plants. They show antioxidant activity and their effects on human nutrition and health is considerable. The mechanisms of action of flavonoids are through scavenging or chelating process.^[24]

Concerning the antioxidative and anticarcinogenic effects of tea, researchers reported that green tea antioxidant (GTA) had antioxidative activity toward hydrogen peroxide and superoxide and that GTA prevented oxygen radical and hydrogen peroxide induced cytotoxicity and inhibition of intercellular communication in cell culture.^[16]

Antimicrobial Activity

The antimicrobial activity of different tea samples are depicted in Table 4.

Table 4: Antimicrobial Activity of tea samples in mm

Tea	<i>Pseudomonas</i>	<i>Proteus</i>	<i>Corynebacterium</i>	<i>Streptococci</i>	<i>E.coli</i>	<i>Bacillus</i>	<i>Klebsiella</i>	<i>Staphylococcus aureus</i>
Tetly	15	17	6	6	8	9	15	9
Lipton	20	10	8	6	9	6	12	6
Organic Tulsi	10	10	7	7	9	7	25	6
Rose	7	4	0	9	6	8	10	7
Lemon	0	6	0	0	4	0	0	3
Gtee	6	6	11	7	7	7	10	6
Pipple	3	10	6	6	9	9	12	10
Lemon Grass	8	7	6	6	8	9	13	7

Results obtained in the present study revealed that the tested eight tea extracts possess antibacterial activity against *Pseudomonas* sp (20mm), *Proteus* sp (17mm) and *Klebsiella* sp (25mm) (Table 4). When tested by the well diffusion method, the tea extracts of Lipton showed significant activity against *Pseudomonas* sp (20mm). Extract of Tetly exhibit highest activity against *P. vulgaris* (17mm) and Organic tulsi showed highest activity against *Klebsiella* (25mm).

CONCLUSION

It can be concluded that the extracts of different tea samples were rich source of 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 transferase (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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