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A COMPARATIVE ASSESSMENT OF PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES BETWEEN LEAF AND DUST OF BLACK TEA EXTRACTS

Shreya Mandal [1], Animesh Samanta [1], Arpita Patra [1], Shrabani Pradhan [1], Suchismita Roy [1], Koushik Das [1], Atiskumar Chattopadhyay [2], Dilip Kumar Nandi [1]*

[1]Department of Nutrition, Microbiology and Human Physiology, Raja N. L. Khan Women's College, Paschim Medinipore, West Bengal, India

[2]Secretary, Faculty Council of Science, Jadavpur University, Kolkata, West Bengal, India\

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***Corresponding author**
E-mail: dilipnandi2004@yahoo.co.in

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ABSTRACT

The present study is designed to investigate the comparison level of antimicrobial, antioxidant properties and as well as the total phenolic content estimation of aqueous extract between dusts and leaf black tea. Antimicrobial assay as well as antioxidant activity of phyto-compounds in both types of black tea was detected by using agar well diffusion and chromatographic technique respectively. Total antioxidant activity and phenolic content were measured by using coloriemetric method. In comparison level the antimicrobial activity and screening of phytochemical compounds like saponins, phenolic compounds & tannins, flavonoids, triterpenoids were significantly ($P<0.05$) different in both aqueous extract of leaf black tea and dust black tea. Total phenolic contents in the leaf black tea were 130.51mg Gallic acid equivalent/g of dried aqueous extract. In conclusion the leaf black tea has potential to be used as a natural antioxidant which is attributed to the rich presence of secondary metabolites and exhibit medicinal as well as physiological activities.

Keywords: Black tea, Phytochemicals, Antimicrobial activity, Antioxidant, Phenolic content, Chromatographic study

INTRODUCTION

Plants are the important source of drugs; especially in traditional medicine. In the world, phytomedicines had been used in past to treat various degenerative diseases long before the introduction of modern medicine. Medicinal plants are important for the treatment and management of human as well as animal diseases due to the presence of phytocompounds. Phytochemicals are naturally occurring compounds which are of great significance in the protection from various chronic diseases[1] and phytotherapy also is a cost effective and less side effect after treatment option. The most important bioactive constituents of plants are steroids, terpenoids, alkaloids, carotenoids, tannins, flavonoids, phenols and glycosides which serve as a valuable starting material for drug development[2].

Natural products can provide unlimited opportunities for the new drug discoveries because of their unmatched chemical diversity. The plant is contained natural active constituents which can be derived from any part of plant like flowers, bark, leaves, seeds, roots, fruits, etc. The useful medicinal effects of plant materials typically result from the combinations of the secondary products present in the plant[3]. Tea stands as the second most non-alcoholic beverage around the world and it is also the less expensive one[4]. Black tea, one of the best known of preparations, is made from *Camellia sinensis*[5]. Tea is generally consumed in the forms of green, black, and oolong tea, all of which are originated from the leaves of the plant *Camellia sinensis*. However the processing that leaves undergo makes different teas. The leaves for black tea are prepared by being fully oxidized before being dried. Tea leaves contain 10-30% of polyphenols including catechins, flavonols, phenolic acids, glycosides and plant pigments[6]. Tea leaves are a good source of polyphenols, specially thearubigins, theaflavins and catechins, a decisive group for their antioxidative activity[7-8]. Tea polyphenols are also known by their antibacterial activity. In general, the antibacterial activity decreases when extent of the tea fermentation process, implies stronger activity in green tea than in black tea[7].There are many number of reports on clinical uses of *Camellia sinensis* in various degenerative disorders particularly in cancer, tumor and diabetes that have shown promising results[9]. So, the present study aims to evaluate that the phytoconstituents, antioxidant and antimicrobial activities are compared between the leaf and dust black teas of aqueous extracts.

MATERIALS AND METHODS

Plant materials and chemicals

The branded leaf and dust black tea were collected from open market at Midnapur district, West Bengal, India. The materials were identified by the taxonomist of the Botany Department at the Raja N. L. Khan Women's College, Midnapore. The voucher specimens (Leaf Tea-DJ/497/07042015/TE, Dust Tea-10013022001897) were deposited in the Department of Botany, Raja N. L. Khan Women's College at Paschim Medidipur, West Bengal. All other chemicals used were of analytical grade and were purchased from HiMedia Laboratories Pvt. Limited (Mumbai, India), SRL (India).

Bacterial strain and culture conditions

Two gram negative and two gram positive indicator bacteria are used for antimicrobial assay respectively *Escherichia coli* (MTCC443), *Kleibsiella Pneumoniae* (MTCC 109), *Staphylococcus aureus* (MTCC 3160), and *Streptococcus mutans* (MTCC890), provided by Microbiological laboratory and clinical detection center Midnapur (Paschim Medinipur,

India). They were cultured in tryptone soy broth or agar (TSB or TSA) in aerobic condition at 37 °C.

Black tea extracts preparation

Black tea extracts were prepared by adding leaf tea (LT) and dust tea (DT) in 25gm to 250mL of boiling water in each conical flask, steeped for 15–20 min. The infusion was cooled at room temperature and then filtered through Whatman No.1 filter paper. Both resulting filtrate were dried in the air, weight and stored in air tight vacuum container for different analysis.

Phytochemicals analysis

Phytochemicals analyses of the test samples were carried out according to standard methods[10-12].

Test for phytosterols- Salkowski reaction
About 0.5 ml chloroform was added to both extracts in test tubes. Then 1ml of Conc. H_2SO_4 was added to it from the sides of the test tube. Appearance of reddish brown colour in chloroform layer indicates presence of phytosterols.

Test for triterpenoids- Liebermann - Burchard's test
Extracts were treated with few drops of acetic anhydride, boiled and cooled and then Conc. Sulfuric acid was added from the sides of the test tube. A brown ring was shown at the junction of two layers and formation of deep red colour and indicates the presence of triterpenoids.

Test for saponins- Foam test
Small amount of both extracts were taken in test tubes with little quantity of water. Shake vigorously. Appearance of foam which is persisting for 10 minutes indicates presence of saponins.

Test for alkaloids- Dragendorff test
Dissolve both the extracts of black teas by chloroform. Evaporate chloroform and acidify the residue by adding few drops of Dragendorff reagent (Potassium Bismuth Iodide). Appearance of orange red precipitation indicates the presence of alkaloids.

Test for carbohydrates- Molisch's test
Mix the extracts with Molisch's reagent and add Conc. H_2SO_4 along the sides of the test tube. Appearance of reddish violet ring the interference indicates the presence of carbohydrates.

Test for flavonoids- Lead acetate test
The alcoholic solution of the extracts was added to few drops of 10% Lead acetate solution. Appearance of yellow precipitation indicates the presence of flavonoids.

Test for phenolic compounds and tannins- Ferric chloride test
Both extracts (each 2ml) was taken in the test tubes and ferric chloride solution was added to it drop by drop. Appearance of bluish black precipitation indicates the presence of phenolic compounds and tannins.

Test for proteins- Ninhydrin test
Few drops of Ninhydrin are added to the both extracts. Appearance of blue colour indicates presence of amino acid whereas proteins may rarely give positive result.

Test for glycosides- Keller-Killiani test
Both extracts were taken in the test tubes, to add 1ml of glacial acetic acid and few drops of ferric chloride solution and also Conc. H_2SO_4 in each extract (Slowly through the sides of the test tube). Appearance of reddish brown ring at the junction of the two liquids indicates the presence of de-oxy-sugars.

Thin layer chromatography analysis for antioxidant constituents

About 2 µg of both extracts of tea were loaded on a TLC plate (Merck, 20 cm x 20 cm). The plate was developed with methanol: chloroform: hexane (7:2:1, v/v/v) to separate various constituents of the extracts. The developed plates were dried by hair drier. Then the antioxidant constituents were analyzed by DPPH technique. For this purpose, 0.05% of methanolic solution of DPPH was sprayed on the surface of developed TLC plates and incubated for 10 min at room temperature. The active antioxidant constituents of both the extracts of tea were detected as yellow colour was produced by the reduction of DPPH in the purple background on the TLC plates. Ascorbic acid was used as standard antioxidant compound[13].

Determination of total phenol content

The amounts of phenol compounds in both the extracts of tea were determined using folin ciocalteu reagent, according to the modified method[14]. 1 ml of the plant extract/standard solution was mixed with 5 ml Folin-Ciocalteu reagent and 4 ml (7.5% sodium carbonate) of sodium carbonate (Na_2CO_3). The tubes were vortexed for few seconds and allowed to stand for 30 min at 20°C for colour development. Absorbance of samples and standard were measured at 765 nm using spectrophotometer against blank. A typical blank solution contained the solvent used to dissolve the plant extract. The total content of phenolic compounds plant extracts in gallic acid equivalents (GAE) was calculated using the following equation:

$$C = (c \times V)/m$$

Where C = total content of phenolic compounds, mg/g plant extract, in GAE, c = the concentration of gallic acid established from the calibration curve (mg/ml), V = the volume of extract in ml, and m = the weight of plant extract in g.

Antioxidant activity determination by DPPH free radical scavenging assay

DPPH free radical scavenging activity of both the extracts of tea was measured by this method[15]. For this analysis, ascorbic acid (Standard) was dissolved in methanol (Sigma-Aldrich) and methanol fractions of TA bark were used as the test solutions. About 1 ml of each fraction was placed into test tubes and 0.5 ml of 1 mmol/L DPPH solution in methanol was added. The test tubes were incubated for 15 min and the absorbance was read at 517 nm. A blank solution contained of DPPH dissolved in same amount of methanol. The DPPH radical scavenging activity percentage was calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [A_{517}\text{control} - A_{517}\text{extract}) / A_{517}\text{control}] \times 100$$

Antimicrobial analysis

The antimicrobial activity was determined in both extracts of tea using agar well diffusion method. The antibacterial activities of both black tea aqueous extracts (concentration of compound 50%, 100 %) were tested against two Gram-positive— *S. aureus, S. mutans* and two Gram-negative— *E. coli and K. pneumoniae*, human pathogenic bacteria; Zone of inhibition of both black tea aqueous extracts were compared with standards like chloramphenicol for antibacterial activity. The results

showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms[16].

Statistical Analysis

The values were expressed as Mean ± SE. Data were analyzed using one-way ANOVA followed by t-test. P value < 0.05 was considered as significant[17]. ANOVA was followed by multiple two-tail t-test and data with different superscripts (a, b) in a specific vertical column differ from each other significantly (P< 0.05). Statistical analysis was performed using SPSS 12.0 and MS-Excel 2007.

RESULTS

The result obtained from gram percent (g %) of aqueous extract of both the leaf and dust black teas were 66g% and 43.6g% respectively as shown in table 1. So, the g% of aqueous extract of leaf of black tea was greater than dust of black tea. The preliminary phytochemicals screening was revealed that the presence of saponins, phytosterols, triterpenoids, alkaloids, carbohydrates, polyphenols, tannins, proteins, glycosides and flavonoids were compared between leaf and dust of black tea of aqueous extracts as shown in the table 2. In this experiment some secondary metabolites (such as saponins, triterpenoids, polyphenols, flavonoids and tannins) were present in high concentration of aqueous extract of black leaf tea than black dust tea.

The plates TLC were developed in methanol: chloroform: hexane (7:2:1, v/v/v) and sprayed with 0.05% DPPH reagent. Purple colour of DPPH reagent was bleached by yellow spots was the indication of positive antioxidant activity. Both the aqueous extracts of dust and leaf of black teas in terms of DPPH free radical scavenging activity showed one resolved TLC band with strong antioxidant activity and another spot with weak antioxidant activity as compared to standard antioxidant ascorbic acid. In this experiment both types of black tea showed antioxidant activities in comparison to standard ascorbic acid (Figure 1). DPPH is a free radical and it gives strong absorption band at 517 nm in the visible region of electromagnetic radiation. As antioxidant compounds donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging activity. The results were compared with that of ascorbic acid and the aqueous extracts of both black teas. The leaf of black tea showed better result than dust of black tea (Figure 2). Total phenolic content of both teas of aqueous extracts was shown in table 4 and figure 3. In this experiment black of leaf tea was contained huge amount polyphenols than dust of black tea. They were significantly (P< 0.05) differ from each other. Antimicrobial activity of both black teas aqueous extracts showed greater result against gram negative bacteria than gram positive bacteria as shown in table 3. In this experiment the aqueous extract of leaf and dust black tea showed that greater inhibition zone against gram negative bacteria than gram positive bacteria. But leaf black tea showed better antimicrobial activity than dust black tea (Figure 4).

Table 1: Percentage of extract from aqueous of the leaf and dust black teas

Black Tea	Amount of Solvent (ml)	Amount of Tea (g)	Amount of extract (g)	Percentage of extract (g %)
BLT	250	25	16.5	66
BDT	250	25	10.9	43.6

BLT**:** Black Leaf Tea, BDT: Black Dust Tea

Table 2: Preliminary phytochemicals analysis of the leaf and dust black teas of aqueous extracts

Sl. No.	Phytoconstituents	Tests	BLT	BDT
1.	Phytosterols	Salkowski reaction	+	+
2.	Triterpenoids	Liebermann - Burchard's test	++	+
3.	Saponins	Foam test	++	+
4.	Alkaloids	Dragendroff's test	+	-
5.	Carbohydrates	Molisch's test	-	-
6.	Flavanoids	Lead Acetate test	++	+
7.	Phenolic Compounds & Tannins	5% $FeCl_3$ Test	++	+
8.	Proteins	Ninhydrin test	-	-
9.	Glycosides	Keller-Killiani test	-	-

BLT: Black Leaf Tea, **BDT:** Black Dust Tea, ++ **:** Highly present, + **:** Slightly present, - **:** Absent

Table 3: Antimicrobial activities of the leaf and dust black tea aqueous extracts and zone of inhibition

	Diameter of Zone of inhibition (mm)* against							
	Staphylococcus aureus		*Streptococcus mutans*		*Klebsiella pneumoniae*		*Escherichia coli*	
	Conc. of compound		Conc. of compound		Conc. of compound		Conc. of compound	
	100%	50%	100%	50%	100%	50%	100%	50%
BLT	15	7.8	17	8.2	23	8.5	35	22
BDT	9.7	4.6	13	5.8	17	5.5	23	10
Chloramphenicol (STD)	18	10	20	12	28	15	45	25

*The zone of inhibition (mm) taken as average, **BLT:** Black Leaf Tea, **BDT:** Black Dust Tea, **STD:** Standard

Table 4: Total phenolic content estimation from the leaf and dust of black tea aqueous extracts (mg GAE/g). Data are expressed as Mean ± SE (n=6)

Black Tea Aqueous Extract	Total phenol contents (mg/g, Gallic Acid Equivalent)
BLT	130.51 ± 0.41^a
BDT	82.42 ± 0.85^b

BLT: Black Leaf Tea, **BDT:** Black Dust Tea

Figure 1: Thin layer chromatography antioxidant activity analysis of Black tea water extracts and Ascorbic acid. TD- Tea Dust, TL- Tea Leaf, STD- Ascorbic acid

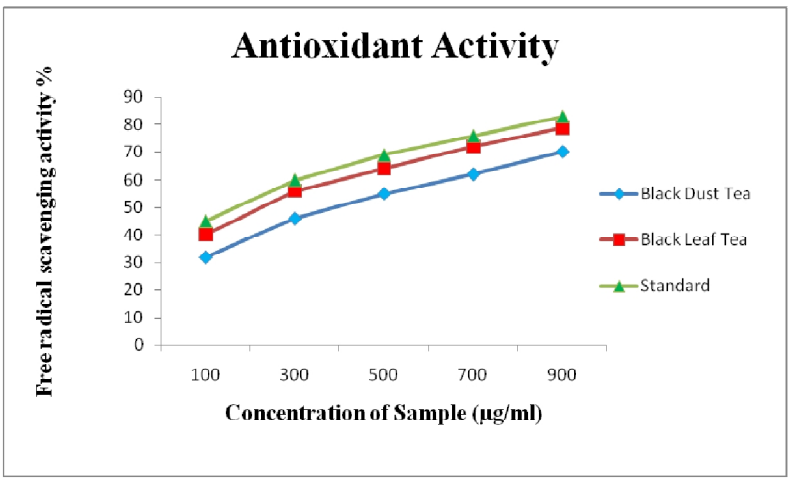

Figure 2: DPPH free radical scavenging activity of Black tea dust and leaf water extracts Standard- Ascorbic acid

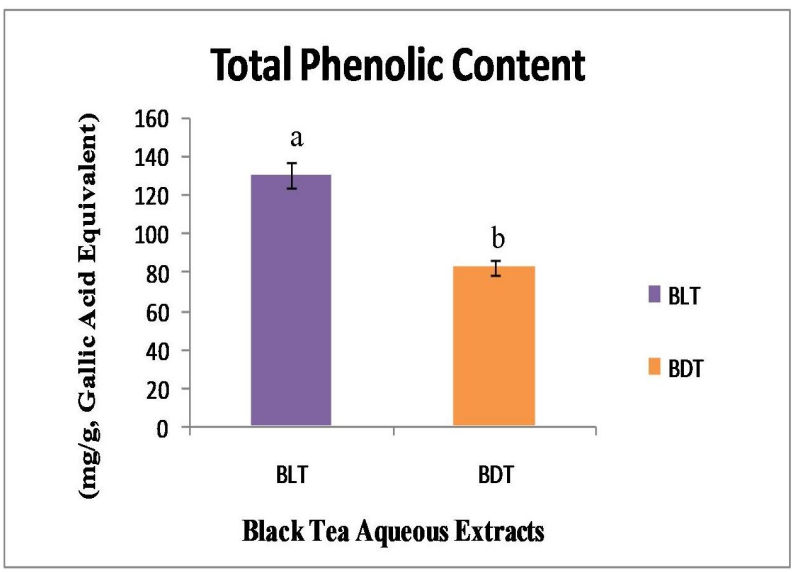

Figure 3: Total phenol contents of the aqueous extracts of BLT and BDT. Data are expressed as mean ± SE (n=6). BLT: Black Leaf Tea, BDT: Black Dust Tea

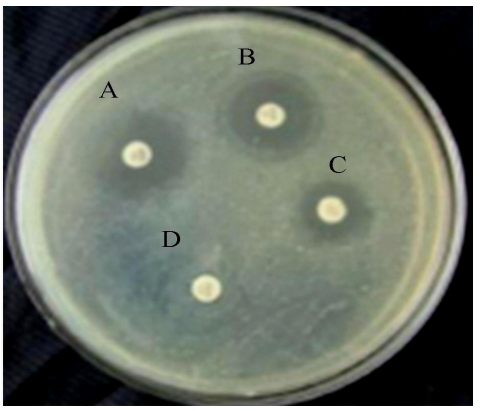

Figure 4: Inhibition zone against Escherichia coli (Indicator microbes of gram negative bacteria)
A-BLT (Black Leaf Tea), B- Chloramphenicol (STD), C- BDT (Black Dust Tea), D- Control (Aqueous)

DISCUSSION

In the present investigation, the obtained gram percent (g%) of aqueous extract of both the leaf and dust black teas revealed that black leaf tea aqueous extract contained huge amount of phytocompounds and the extracted quantity of black leaf tea was greater than dust black tea. Physicochemical standardization is the most prominent means for quality assurance of herbal products[18]. Phytochemical analysis conducted on the tea extracts revealed the presence of constituents which are known to exhibit herbal medicinal as well as physiological activities[19]. The aqueous extract of leaf of black tea showed high concentration of saponins, triterpenoids, polyphenols, flavanoids and tannins than aqueous extract of dust of black tea. Due to presence of huge amount of phytoconstitutes in leaf of black tea, it showed higher antioxidant activity than dust of black tea. This antioxidant activity result in both teas extracts was shown in figure 1 and 2. This is related to the fact that antioxidants can prevent free radicals, primarily highly reactive oxygen and nitrogen species, from damaging human health. Harmful effects of free radicals and oxidative stress can be reduced by regular consumption of tea like beverages which is exhibits antioxidant activity[20]. Phytochemicals are known to be synthesized by plants in response to different microbial infection and they have been found to be different antimicrobial substances against the wide array of micro-organisms in-vitro and their activities are probably due to their ability to complex with extracellular and soluble proteins and to complex with the bacterial cell wall[21]. The antimicrobial activity of leaf and dust of black tea was detected against four entero-pathogenic bacterial strains: *Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumonia, Escherichia coli,* and compared to that of reference Standard drug Chloramphenicol disc. Aqueous extract of both teas showed inhibition against tested strains to varying degrees of 100% and 50% concentrations. The black tea leaf (BLT) of aqueous extract showed maximum inhibition against tested strain. Thus, the aqueous extract of BLT has shown great antioxidant and antimicrobial activities. Phenolic compound embrace a broad range of plants secondary metabolite which have health favourable properties. The accumulation of laboratory and clinical studies suggested that polyphenol-rich plants have health promoting effects with respect to metabolic health[22] and cancer prevention[23]. Polyphenols are natural antioxidant from plants and are consumed in the forms of vegetables, fruits and beverage such as tea, coffee and wine[24]. The known in-vitro antioxidant properties of catechins and other polyphenolic compounds of tea have led to interest in the potential health benefits of tea consumption[25,26]. In this experiment, there is a significant ($P<0.05$) difference between in leaf and dust of black tea aqueous extracts. Black leaf tea contained more amount of phenolic content than black dust tea. This result denoted that the leaf of black tea possess polyphenols, chiefly responsible for antioxidant activity. It was mentioned that the presence of phenolic content could show the antioxidant and free radical scavenging properties which was confirmed by our experiment. According to our investigation, the high contents of polyphenol in the leaf of black tea can explain its high free radical scavenging activity. The results obtained from the above experiment showed that leaf of black tea contains different major antioxidative compounds in high concentration which may be helpful for treatment of diseases as well as for suppressing the growth of many pathogenic organisms.

CONCLUSION

It has been shown that leaf of black tea consists of many useful compounds such as flavonoids, tannins, phytosterols, saponins, polyphenols and alkaloids. Its antioxidant activity is largely due to the presence of polyphenols. Tea polyphenols or total phenolic contents are well-known for their antioxidant properties. The antioxidant and antimicrobial properties of black leaf tea are responsible on presence of large amount of phytocomponents. So the results further support the view that the black leaf tea is a promising source of natural useful therapeutic agents. Further, there is a need to isolate, purify and identify these natural active constituents present in leaf black tea as an important medicinal plant, which is extensively used in Indian system of medicine for prevention of degenerative diseases.

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