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Chemical Profiling of Black Tea Polyphenols

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Abstract: In present project locally grown black tea (Qi-men variety) was explored for its nutritional and antioxidant potential. Furthermore, polyphenols extraction was carried out by using water, ethanol and methanol as solvent at varying time conditions. Proximate profiling indicating moisture, crude fat, crude protein, crude fiber, ash and NFE as 7.01±0.20, 4.51±0.06, 15.12±0.51, 15.33±1.52, 4.86±0.08 and 53.17±1.97%, respectively. Likewise, tested sample exhibited good mineral status dominated by potassium and sodium. The different antioxidant indices were affected significantly by time and solvents. In this context, ethanol and 60 min extraction time showed maximum TPC, FRAP, DPPH and beta-carotene activity as 1150.92±15.01 and 833.33±1.12 mg/100g GAE, 754.44±12.30 and 609.89±16.04 µmolFe^{2+}/g, 71.13±3.20 and 65.32±3.26 and 67.74±3.10 and 66.24±3.90%, respectively whilst minimum amount of these attributes were recorded in water extract and 30 min extraction time by 354.02±5.12 and 656.44±11.05 mg/100gGAE, 471.78±12.50 and 558.00±14.03 µmol Fe^{2+}/g, 55.27±3.14 and 59.69±2.30 and 48.27±3.90 and 61.30±2.90%, correspondingly. Likewise, theaflavin, thearubigins, catechins and caffeine contents were detected highest in ethanolic extract 2.55±0.25, 20.93±1.10, 1.90±0.02 and 1.89±0.01% followed by methanol 2.05±0.25, 19.45±1.8, 1.68±0.03 and 1.72±0.06% and water extracts 1.23±0.25, 17.69±1.30, 1.52±0.04 and 1.54±0.03%, respectively. Among the time intervals, 60 min proved more efficient for the extraction of these bioactive moieties than that of 90 and 30 min. Conclusively, the tested black tea extracts holds promising antioxidant status thus prove as potential candidates against lifestyle related disorders.

Key words: Theaflavin, thearubingins, polyphenols, extraction time, DPPH activity

INTRODUCTION

Recently, diet based therapy with special reference to polyphenols has been invigorated worldwide and people are using natural food materials as an intervention against various maladies. Among different dietary regimen tools, polyphenolic enriched functional and nutraceutical foods engrossed attention due to their acceptability, easy access, low cost and long administration safety (Thielecke and Boschmann, 2009). Functional/nutraceutical foods are developed from different sources nevertheless, plant based products leading the issue due to their rich phytochemistry that in turn ameliorates different ailments (Rains *et al*., 2011). Black tea (*Camellia sinensis*) member of *Theaceae* family is one such example of plants containing bioactive molecules with unique nutraceutical potential (Wang *et al*., 2011). Globally, tea is a popular beverage after water made from tea plant leaves. The historians have linked its consumption almost 5000 years back (Yang *et al*., 2009). Tea is mainly divided into three distinct types i.e., black, green and oolong differed in terms of processing method and chemical profile. Green tea accounts for approximately 20% of total tea production, consumed primarily in East and South East Asia. Contrarily, black tea that occupies approximately 78% of the world share is consumed mainly in North America, Europe and North Africa. During manufacturing of black tea, leaves are crushed and subjected to enzymatic oxidation process called fermentation (Hsu *et al*., 2010). Subsequent oxidative condensation of the catechins leads to the production of theaflavin (benzotropolone dimers of catechins) as well as higher molecular weight polymers i.e., thearubigins. Both constituents are responsible for the specific taste and color of the black tea. Polyphenolic compounds including substantial amount of flavonoids dominate the typical composition of black tea (Wang *et al*., 2012).

Flavonoids are further classified in to six groups based on structure and position of the heterocyclic oxygen carbon oxygen ring i.e., flavones, flavanones, isoflavones, flavanols, flavonols and anthocyanins. Flavanols consist of unoxidized catechins including theaflavin (TF) and thearubigins (TR) whilst flavonols are comprised of qureicitin, myricetin and kaempferol. Tea is also a good source of phenolic acid, caffeine, theobromine, theophylline and flavor compounds enriched with linalool (Dimpfel *et al*., 2007). Additionally, tea carries unique amino acid, theanine that has been extensively investigated for biological activity. Tea composition is likely to be associated with origin, fermentation conditions and processing (Baptista *et al*., 2012).

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Isolation of functional ingredients is a delicate process because components of interest are often form complex with other food matrixes like sugars and proteins. They are also vulnerable to oxidation, little fluctuation in processing parameters resulting subsequent degradation (Tura and Robards, 2002). The solvent partition method is a promising technique for separating tea nutraceuticals by optimizing the factors like solvent, time and temperature. For the extraction of tea polyphenols, besides water other solvents like ethanol, methanol, acetonitrile and acetone are commonly in practice (Sun and Ho, 2005). Nevertheless, methanol, ethanol and acetonitrile are performed better after optimizing extraction time and temperature (Gong *et al*., 2012).

Black tea is considered as a dietary source of antioxidant nutrients like theaflavins and thearubigins along with unoxidized catechins. By virtue of their singlet oxygen quenching ability, they act as safeguard against oxidative stress there by effective in the maintenance of cardiac health and cancer care. The mechanistic approach of these antioxidants is likely to be associated with inhibition of redox sensitive transcription factors and pro-oxidant enzymes such as xanthine oxidase or nitric oxide synthase. However, their involvement in antioxidative enzyme induction as in glutathione-S-transferases is also well documented. Black tea polyphenols act as preventive agent against numerous physiological disorders by disrupting electron chain thus inhibit the progression of various ailments (Amić and Lučić, 2010; Phung *et al*., 2010).

In current study, locally grown variety of black tea (Qi-men) was explored for its nutritional composition. Moreover, optimization of polyphenol extraction under varying time and solvents was the limelight of the study.

MATERIALS AND METHODS

Black tea variety (Qi-Men) was procured from the National Tea Research Institute (NTRI), Shinkiari, Mansehra. The reagents (analytical and HPLC grade) and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

Characterization of black tea: Initially, black tea was analyzed for various compositional traits including proximate assay, mineral profile, alkaloids and polyphenols extraction.

Compositional analysis: Black tea samples were investigated for moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract in triplicate on dry weight basis.

Moisture content: Moisture content of black tea was measured by drying sample in Air Forced Draft Oven (Model: DO-1-30/02, PCSIR, Pakistan) at 105±5°C till constant weight following the procedure of AACC (2000) Method No. 44-15A.

Crude protein: Crude protein content was estimated by using Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany) as described in AACC (2000) Method No. 46-30.

Crude fat: Soxtec System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) was used to determine the crude fat content in tea sample following the guidelines mentioned in AACC (2000) Method No. 30-25.

Crude fiber: Fiber in the fat free sample was assessed by digesting first with 1.25% H_2SO_4 for 30 min followed by 1.25% NaOH solution using Labconco Fibertech (Labconco Corporation Kansas, USA) as mentioned in AACC (2000) Method No. 32-10.

Total ash: Ash was calculated by using Muffle Furnace (MF-1/02, PCSIR, Pakistan). After charring, incineration was done at 550°C till grayish white residue (AACC, 2000; Method No. 08-01).

Nitrogen Free Extract (NFE): NFE was calculated through subtraction method following the expression:

$$NFE\,(\%) = 100 - (Moisture + CP + Crude\,fat + Crude\,Fiber + Ash)\,(\%)$$

Where CP = Crude protein

Minerals: Black tea sample was probed for mineral profile after wet digestion considering the guidelines of AOAC (2006). For the assessment of sodium and potassium, Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge) was used. Likewise, Atomic Absorption Spectrophotometer (Varian AA240, Australia) was run for the measurement of calcium, iron and manganese.

Alkaloids: In tea sample, total alkaloids comprised of caffeine and theobromine were measured following the protocol of AACC (2000).

Polyphenols extraction: Tea polyphenols were extracted by following the protocol of Rusak (2008) using solvents including water, ethanol and methanol at three different time intervals 30, 60 and 90 min on constant temperature of 60°C (Table 1). Resultant extracts were filtered using vacuum filtration assembly and solvents were recovered by Rotary Evaporator (EYELA, N-N series, Japan) at 40°C. The yield of respective sample was calculated and stored at 4°C until further use.

The resultant extracts were subjected to different assays as described below.

Total polyphenols: Total phenolics of resultant extracts were estimated spectrophotometricaly using Folin-Ciocalteau method (Singleton *et al*., 1999). The extract

Table 1: Treatments used for estimation of extraction efficiency

Treatments	Solvent	Time (min)
T_1	Water	30
T_2	Methanol	30
T_3	Ethanol	30
T_4	Water	60
T_5	Methanol	60
T_6	Ethanol	60
T_7	Water	90
T_8	Methanol	90
T_9	Ethanol	90

(125 µL) was mixed with 125 µL of Folin-Ciocalteau reagent along with 500 µL of distilled water and allowed to stand for 5 min at 22°C. Following resting period, 4.5 mL of sodium bicarbonate solution (7 %) was added to the mixture. After 90 min, absorbance was measured at 765 nm using a UV/vis Spectrophotometer (CECIL CE7200) against control. Total polyphenols were calculated and expressed as gallic acid equivalent (mg gallic acid/100g).

Antioxidant activity: Total antioxidant activity of the extracts was monitored using assay based on coupled oxidation of beta-carotene and linoleic acid (Taga *et al*., 1984). Briefly, beta-carotene 2 mg was dissolved in 20 mL chloroform, 40 mg linoleic acid and 400 mg Tween 20. After removing chloroform, 3 mL of the prepared emulsion was added in 0.10 mL sample and placed in a water bath for 120 min. Oxidation of beta-carotene was determined spectrophotometricaly at 470 nm.

Free radical scavenging activity (DPPH assay): DPPH radical scavenging activity was measured according the procedure of Brand-Williams *et al*. (1995). For the purpose, one mL of DPPH was added to each extract (4 mL) and incubated at room temperature for 30 min. The absorbance was noted at 520 nm using Spectrophotometer (CECIL CE7200). Percent inhibition was calculated using the following formula:

$$\text{Reduction of absorbance (\%)} = [(AB - AA) / AB] \times 100$$

AB = Absorbance of blank sample (t = 0 min)
AA = Absorbance of tested extract solution (t = 30 min)

Ferric Reducing Antioxidant Power (FRAP): The FRAP test was performed according to the method of Sun *et al*. (2010). Tea extract (0.5 mL) was mixed with phosphate buffer (1.25 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1.25 mL, 1%). After incubation, 10% TCA (1.25 mL) along with 0.1% ferric chloride were added in the mixture and then left at room temperature for 10 min. Sample absorbance was measured at 700 nm.

Theaflavin and thearubigins estimation: Estimation of theaflavin (TF) and thearubigins (TR) of the resultant extracts was carried out following the protocol of Angayarkanni (2002). Briefly, in a separating funnel equal amount of extract and Iso-butyl Methyl Ketone (IBMK) were added. After separation, resultant organic layer was diluted with 9 mL of ethanol, absorbance (380 nm) was calculated and considered as A. In the next step, 10 mL of organic phase was diluted by adding 10 mL of Na_2HPO_4 (2.5%). The separated layer was again diluted with ethanol, measured absorbance at same wavelength and termed as B. Lastly, butanol treated aqueous phase was eluted with 9 mL of ethanol and measured absorbance at 380 nm, named as C.

$$TF (\%) = 4.313 \times C$$

$$TR (\%) = 13.643 \times (A+C-B)$$

Catechins determination: Total catechins in the extracts were estimated through vanillin-HCl method using UV/vis Spectrophotometer (CECIL CE7200) at 500 nm (Ayumiko *et al*., 2003).

Caffeine determination: A 25 mL of tea extract was dissolved in distilled water (25 mL). The solution was stirred for one hr using magnetic stirrer and heated gently to remove caffeine. Finally, the absorbance of caffeine layer was measured at 310 nm against corresponding blank (Belay *et al*., 2008).

Statistical analysis: Data were obtained by applying Completely Randomized Design (CRD) and further subjected to statistical analysis using Statistical Package (Costat-2003, Co-Hort, v 6.1.). Levels of significance were determined (ANOVA) using 2-factor factorial CRD following the principles outlined by Steel *et al*. (1997).

RESULTS AND DISCUSSION

Compositional analysis of black tea: Proximate composition is a key factor for assessing the quality of raw material. Black tea (dry weight basis) was subjected to different quality traits assessment and revealed moisture, crude fat, crude protein, crude fiber, ash and NFE as 7.01±0.20, 4.51±0.06, 15.12±0.51, 15.33±1.52, 4.86±0.08 and 53.17±1.97 %, respectively (Table 2).

The results are in line with the earlier findings of Mohammed and Sulaiman (2009), they narrated moisture, crude fat, crude protein, crude fiber, ash and caffeine from 5.60 to 7.50, 3.56 to 5.61, 14.25 to 21.22, 14 to 18.82, 4.90 to 7.20 and 1.98 to 4%, respectively. Later, Heong *et al*. (2011) probed black tea samples for moisture, protein, fat, ash, total amino acid and polysaccharides contents and reported values as 7.47, 27.47, 2.12, 5.45%, 34.62 L-glutamic acid/100mL, 30.17 mg glucose/100mL and 24.35 mg/100mL, respectively. Likewise, Imran *et al*. (2011) carried out the proximate profiling of different Pakistani black tea brands and

revealed moisture, crude fat and ash from 6.9-7.08, 4.23-7.67 and 4.18-5.11%, respectively. Earlier Wu and Bird (2010) observed 15-25% protein, 16-30% fiber and 5-10% of ash in different black tea samples.

Total alkaloids were estimated as 2.55±0.05% on dry weight basis (Table 2). The result for alkaloids was comparable with the previous findings of Erol *et al.* (2010), they observed total alkaloids in the range of 25.97 to 26.26 mg/g in different Turkish black tea samples. Likewise, Chee and Juneja (1997) detected 1.9 to 3.5% of total alkaloids in different green and black tea samples. In a similar research, Xie *et al.* (1993) also estimated a value of 2.5 to 5.3% for this trait. The compositional disparities in black tea regarding proximate and alkaloids are due to varietals variations, climatic conditions, topographic locations and agronomic practices. Moreover, the maturity of leaf and the stage of leaf picking are also the factors of prime importance.

Table 2: Compositional analysis of black tea

Components	Quantity (%)
Moisture	7.01±0.20
Crude fat	4.51±0.06
Crude protein	15.12±0.51
Crude fiber	15.33±1.52
Ash	4.86±0.08
NFE	53.17±1.97
Total alkaloids (%)	2.55±0.05

Values are expressed as means ± standard deviation

Table 3: Mineral profile of black tea

Minerals	(mg/100g)
Potassium	1892.5±75.77
Calcium	330.53±19.65
Sodium	7.67±0.38
Manganese	66.90±3.83
Iron	20.38±0.76
Zinc	4.19±0.12

Values are expressed as means ± standard deviation

Mineral profile in the current study (Table 3) comprised of potassium, calcium, sodium, manganese, iron and zinc and their respective values were 1892.50±75.77, 330.53±19.65, 7.67±0.38, 66.90±3.83, 20.38±1.76 and 4.19±0.12 mg/100g. The results for Mn, Fe, Cr and Zn are in accordance with the earlier findings of Cabrera *et al.* (2006) who detected these minerals in different tea samples that varied from 50.6 to 371.4, 7.6 to 9.87, 4.8 to 11.4 and 56.3 to 78.6 mg/100g, respectively. However, the results for sodium, calcium and potassium are in line with the work of McKenzie *et al.* (2010), examined variations in Na, K and Ca from 5.00 to 25.5, 1535 to 11351 and 235 to 2526 mg/100g, respectively. Earlier, Chee and Juneja (1997) determined K, Ca, Na and Fe in different tea samples that ranged from 1810-2795, 354-652, 3-9 and 10.4-38 mg/100g, respectively. Recently, Szymczycha-Madeja *et al.* (2012) evaluated different black tea samples for mineral contents and found Al, Ca, K, Mg, Mn and Na as 50-2700, 320-460, 80-2770, 90-3400, 81-213 and 1-12.5 mg/100g, respectively. Earlier, Gallaher *et al.* (2006) noted higher K (82.16±2.32 mg/Kg) and Ca (22.90±1.12 mg/kg) as compared to Na (7.11±0.21 mg/kg) in ten commercial black tea samples. In a study, Chand *et al.* (2011) observed variations among mineral contents and ascribed these as a function of climate, soil and agronomic practices.

Extracts analysis: Mean squares in Table 4 indicated that antioxidant indices of black tea extracts were significantly affected by solvents and time intervals. However, their interactive effect showed non-momentous trend except for TPC, FRAP and beta-carotene. Means for the effect of solvents (Table 5) exposed that the highest TPC 1150.92±15.01mg/100g GAE was recorded in ethanol followed by methanol 722.41±11.23 while the lowest 354.02±5.12 mg/100gGAE in water extract. Likewise, maximum FRAP value (754.44±12.30 µmol Fe^{2+}/g) was exhibited by

Table 4: Mean squares for antioxidant indices of tea extracts

SOV	df	TPC	FRAP	DPPH	beta-carotene	Theaflavin	Thearubigins	Catechins	Caffeine
Solvent (A)	2	70563**	232985**	339.682**	45.4909**	3.96480**	41.7450**	0.32203**	0.32940**
Time (B)	2	1431522**	6176**	13.036**	2.9101**	0.21325**	0.6268**	0.01428**	0.00556*
A x B	4	11492**	1303**	1.376^{NS}	0.3652**	0.01561^{NS}	0.2645^{NS}	0.00304^{NS}	0.00309^{NS}
Error	18	454	6	0.842	0.0380	0.01075	0.2601	0.00282	0.00104

* = Significant
** = Highly significant
NS = Non significant

Table 5: Effect of solvents on antioxidant potential of tea extracts

Parameters	Ethanol	Methanol	Water
TPC mg/100g GAE	1150.92±15.01a	722.41±11.23b	354.02±5.12c
FRAP µmol Fe^{2+}/g	754.44±12.30a	569.78±13.01b	471.78±12.30 c
DPPH (%)	71.13±3.20a	62.37±2.01b	55.27±3.14c
Beta-carotene (%)	67.74±3.10a	60.87±3.20b	48.27±3.90c
Theaflavin (%)	2.55±0.25a	2.05±0.25b	1.23±0.25c
Thearubigins (%)	20.93±1.10a	19.45±1.80b	17.69±1.30c
Catechins (%)	1.90±0.02a	1.68±0.30b	1.52±0.40c
Caffeine (%)	1.89±0.01a	1.72±0.06b	1.54±0.03c

Table 6: Effect of time on antioxidant potential of tea extracts

Parameters	30min	60min	90min
TPC mg/100g GAE	656.44±11.05c	833.33±1.12a	737.56±10.21b
FRAP µmol Fe^{2+}/g	558.00±14.03c	609.89±16.04a	590.22±19.03b
DPPH (%)	59.69±2.30c	65.32±3.26a	62.76±4.85b
Beta-carotene (%)	61.30±2.90c	66.24±3.90a	63.33±3.20b
Theaflavin (%)	1.77±0.03c	2.07±0.06a	1.98±0.58b
Thearubigins (%)	17.72±1.40c	20.48±1.80a	18.97±1.70b
Catechins (%)	1.64±0.01c	1.74±0.03a	1.68±0.01b
Caffeine (%)	1.67±0.03b	1.71±0.06ab	1.77±0.09a

ethanol followed by methanol (569.78±13.01 µmol Fe^{2+}/g) and minimum output (471.78±12.50 µmol Fe^{2+}/g) in water extract. The values for DPPH and beta-carotene were also highest in ethanol and methanol as compared to water as 71.13±3.20 and 67.74±3.10%, 62.37±2.01 and 60.87±3.20% and 55.27±3.14 and 48.27±3.90%, respectively. Means for solvents regarding theaflavin, thearubigins, catechins and caffeine (Table 5) indicated highest values for ethanolic extract as 2.55±0.25, 20.93±1.10, 1.90±0.02 and 1.89±0.01%, respectively. The methanolic extract had values 2.05±0.25, 19.45±1.8, 1.68±0.03 and 1.72±0.06%, respectively for these traits. Likewise, the lowest values were recorded in water extract 1.23±0.25, 17.69±1.30, 1.52±0.04 and 1.54±0.03%, respectively.

The means for the effect of time (Table 6) showed maximum TPC (833.33±18.12 mg/100g GAE), FRAP (609.89±16.04 µmol Fe^{2+}/g), DPPH (65.32±3.26%), beta-carotene (66.24±3.90%), theaflavin (2.07±0.16%), thearubigins (20.48±1.80%), catechins (1.74±0.03%) except for caffeine (1.71±0.06%) in the resultant extracts at 60 min. However, the extracts at 90 min showed maximum caffeine (1.77±0.09%). The extracts at 30 min reflected minimum values for TPC, FRAP, DPPH, beta-carotene, theaflavin, thearubigins, catechins and caffeine by 656.44±11.05 mg/100g GAE, 558.00±14.03 µmol Fe^{2+}/g, 59.69±2.30%, 61.30±2.90%, 1.77±0.03%, 17.72±1.40%, 1.64±0.01% and 1.67±0.03%, respectively. The results of present study are supported by the findings of Luximon-Ramma *et al.* (2005), they noted FRAP values ranged from 357 to 927 µmol Fe^{2+}/g, while, total phenolic varied from 400 to 1200 mg/100g GAE. Likewise, Almajano *et al.* (2008) determined total phenolic contents of black tea 1844 mg/100g GAE. Recently, Chen *et al.* (2012) demonstrated antioxidant activity of different black tea samples through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Total Phenolic Contents (TPC) assay. The observed values for respective traits were 75% and 3000.10 mg/100g GAE. Earlier, Imran *et al.* (2011) examined the antioxidant potential of black tea water extracts through DPPH and ß-carotene assay, the result for the tested parameters varied from 42.01 to 51.36 and 40.02 to 48%, respectively. Previously, Turkmen *et al.* (2006) compared different solvents like water, methanol, ethanol, dimethylformamide (DMF) and acetone for total phenolics and free radical scavenging activity of black tea samples. In the experiment ethanolic extract exhibited the highest polyphenols 1300.30 mg/100g GAE as compared to methanol 820.30 mg/100g GAE, whilst, water showed the least TPC 330.30 mg/100gGAE. The DPPH values for ethanol, methanol and water were 68.9, 58.3 and 29.9%, respectively. Afterwards, Shalini and Sudha (2010) assessed DPPH inhibition of the ethanolic and methanolic extracts of black tea by 97.70 and 95.55%, respectively. Besides, Turkmen *et al.* (2007) explicated a linear association between time and polyphenol extraction yield. Similarly, Jayasekera *et al.* (2011) also reported a significant effect of time on the DPPH and FRAP activity and deduced that polyphenolic yield was dependent on the solvent and extraction time.

The black tea holds higher antioxidant activity owing to the presence of vicinal dihydroxy and trihydroxy components that quench metal ions and inhibit the production of free radicals. Moreover, structural variations in bioactive moieties permit electron delocalization thereby ensure free radical scavenging activity (Khan and Mukhtar, 2007; Chen *et al.*, 2012).

The results regarding theaflavin, thearubigins, catechins and caffeine are corroborated with the work of Imran *et al.* (2011), reported values for these attributes 1.71-1.79, 17.05-23.25, 1.01-1.28 and 1.22-1.25%, respectively in commercial black tea brands. Likewise, Muthumani and Kumar (2007) have elucidated a positive correlation between time and extraction efficiency of black tea bioactive moieties. Theaflavin and thearubigins were estimated as 1.2 to 3.6 and 12.03 to 19.23% during 10 to 60 min extraction time. One of their peers, Yao *et al.* (2006) depicted variations in theaflavin and thearubigins contents from 2-5 and 15-23%, respectively. They expressed that the level of theaflavin and thearubigins were affected by extraction time, temperature and fermentation stage. Previously, Lin *et al.* (2003) documented 2.6-4.8 and 1.23-2.01% of caffeine and catechins, respectively in ethanolic extract of black tea samples.

From the above discussion, it is deduced that the antioxidant characteristics of black tea extracts are affected by the type of solvent and extraction time. In

general, all the extracts showed increased antioxidant activity when extraction time was enhanced from 30 to 60 min however, at 90 min a declining tendency was observed. Similarly, the ethanolic extract performed better than that of methanolic and water extract.

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