Asian Journal of Chemistry Vol. 15, No. 2 (2003), 1121–1126

Comparative Antioxidant Activity and Brine Shrimp Lethality of Green Tea and Black Tea Extracts†

ALLURI V. KRISHNARAJU, DODDA SUNDARARAJU, TAYI V.N. RAO, MUNABAGAL VANISREE and GOTTUMUKKALA V. SUBBARAJU*
Laila Impex R&D Centre, Unit-I, Phase-III, Jawahar Autonagar, Vijayawada- 520 007, India
E-mail: subbaraju@netlinx.com

Aqueous extracts of green tea and black tea have shown strong antioxidant activity in nitro blue tetrazolium (NBT) photoreduction method and 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity. Green tea extracts, however, showed better antioxidant activity compared to the extracts of black tea. The antioxidant activity of these extracts has increased further in ethyl acetate soluble fractions. The activity corroborated well with the content of polyphenols present in these extracts. Aqueous extract of green tea has also shown brine shrimp lethality.

Key Words: Antioxidant activity, Brine shrimp lethality, Green tea, Black tea.

INTRODUCTION

Camellia sinensis (Linn.) O. Kuntze, commonly known as tea, is a multiple branched slow growing shrub or small tree that grows up to 1–3 m. The leaves are usually 4–10 cm long, simple, alternate, erect, with a short petiole; leaf blade is leathery dark glossy green above and light green with hair below. The flowers grow short pedicled and solitary or stalked usually 2–4 in the leaf axils[1]. A species indigenous to China and wildly cultivated in India, Nepal and other Asian countries[2].

The young leaves are plucked and processed into different grades of tea[3]:

Black tea: Process of black tea includes four principal operations, withering (removal of moisture), rolling (twisted between rollers to damage cells suitable for initiation of enzymatic oxidation), fermentation (fermented for 2–4 h which leads to enzymatic oxidation) and drying (dried for 30–40 min at 82–93°C and the final product contains 2–3% moisture).

Oolong tea: It is produced by wilting the fresh tender leaves in the sun, then brushing them slightly and partially fermented.

Green tea: It is processed by using fresh tender leaves and eliminating 2 steps (withering and fermentation) in the above process of black tea. Leaves are directly heated, rolled and dried.

†Laila Impex Communication # 18.

Chemical constituents

Green tea: *Catechins:* (+)-Catechin (+)-gallocatechin, (–)-epicatechin, (–)-epicatechin gallate, (–)-epigallocatechin and (–)-epigallocatechin gallate.

Alkaloids: Caffeine and threanine and other polyphenols.

Black tea: *Purine alkaloids:* Caffeine, theobromine and theophylline.

Triterpene saponins: Theafolia saponins and aglycones including, among others, barringtogenol-C, R1-barringtogenol.

Theaflavins: Theaflavin, isotheaflavin, neotheaflavin, theaflavin-3′-gallate, theaflavin-3-gallate, theaflavin-3,3′-digallate, theaflavic acid, epitheaflavic acid, epitheaflavic acid-3′-gallate and theaflavic acid-3-gallate[1–4].

Flavonoids: These are common to green and black tea and include quercetin, kaempferol, myrecetin, etc. Also contains carotene, riboflavin, nicotinic acid, pantothenic acid and ascorbic acid[1–4].

Traditional uses: Tea is used for stomach disorders, vomiting, diarrhoea and inflarnnnation[1, 2]. It is also used as antioxidant, smooth muscle relaxant, improves ability of skeletal muscles to withstand prolonged strenuous exercise and CNS stimulant[5].

Free radicals and their metabolites which are formed in the body as a consequence of normal metabolic reactions, exposure to pollutants and UV radiation are increasingly recognised for their contribution to tissue injury and degenerative diseases, tumor promotion and carcinogenesis[6, 7]. The present study was undertaken to evaluate the comparative antioxidant activity (superoxide radical scavenging activity and DPPH radical scavenging activity) of fermented and unfermented tea.

Brine shrimp lethality (BSL) assay is a simple bench top bioassay developed by McLaughlin, *et al.*[8, 9], and the results obtained by this assay have been reported to be corroborative with the cytotoxicities determined in 9KB and 9PS cells[10, 11]. We have also investigated the brine shrimp lethality of various extracts of green tea and black tea and the results are presented in this paper.

EXPERIMENTAL

Nitro blue tetrazolium chloride (NBT), 1,1-diphenyl-2-picry hydrazyl (DPPH) and gallic acid were obtained from Sigma Chemicals Co., St. Louis, MO 63178 USA. Riboflavin, sodium dihydrogen orthophosphate, disodium hydrogen phosphate-2-hydrate, EDTA, NaCN, NaOH, methanol, ascorbic acid and other reagents of AR grade were procured from Qualigens Fine Chemicals, Mumbai, India.

Extraction: Green tea raw material (250 g) was extracted with water (2 L × 4), at 110°C for 30 min. All the extracts were combined, filtered through celite and concentrated to half the volume (4 L) under vacuum. A sample of 500 mL of the concentrate was dried in a vacuum drier to a dry powder (GT aq. ext., 9.7 g). The remaining part (3.5 L) of the concentrated extract was partitioned with ethyl acetate (2 × 1 L). Ethyl acetate solubles and insolubles were evaporated, separately, to dry powder (43.5 g and 23.8 g respectively).

Black tea raw material (1 kg) was extracted with water (5 L × 4), at 100°C for

30 min. All the extracts were combined, filtered through celite and concentrated to half the volume (10 L) under vacuum. A sample of 1 L of the concentrate was dried in a vacuum drier to dry powder (BT aq. ext. 10.6 g). The remaining part (9 L) of the concentrated extract was partitioned with ethyl acetate (2 × 3 L). Ethyl acetate solubles and insolubles were evaporated separately to dry powder (52.0 g and 33.7 g respectively).

Determination of superoxide radical scavenging activity: Superoxide radical scavenging activity of various extracts of green tea and black tea was determined by the method of McCord and Fridovich[12]. The assay mixture contained EDTA (6.6 μM) containing 3 μg NaCN, riboflavin (2 μM), NBT (50 μM), various concentrations of test substances (green tea and black tea extracts) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The tubes were mixed well and optical density was measured at 560 nm. The tubes were uniformly illuminated with an incandescent lamp for 15 min and the optical density was measured again at 560 nm. The percentage inhibition of superoxide radical generation was measured by comparing the absorbance values of control and those of the test substances. IC_{50} values were obtained from the plot drawn: concentration (μg) *vs.* percentage inhibition.

Determination of DPPH free radical scavenging activity: DPPH radical scavenging activity was measured by the method of Lamaison *et al.*[13], based on the reduction of methanolic solution of the coloured DPPH. Free radical scavenging ability of the test substances added to the methanolic solution of DPPH is inversely proportional to the difference in initial and final absorption of DPPH solution at 517 nm. Drug activity is expressed as the 50% inhibitory concentration (IC_{50}). The reaction mixture contained 1×10^{-4} mM methanolic solution of DPPH and various concentrations of the test substances. Percentage inhibition was determined by comparing the absorbance values of test and control tubes. IC_{50} values were obtained from the plot drawn: concentration (μg) *vs.* percentage inhibition.

Determination of brine shrimp lethality: Brine shrimp (*Artemia salina*) napuli were hatched using brine shrimp eggs in a conical shaped vessel of 1 L volume filled with sterile artificial sea water (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1 N NaOH) under constant aeration for 48 h. After hatching, 10 napuli were drawn through a pipette and placed in each vial containing 4.5 mL sea water and added various concentrations of drug solutions and volume was made up to 5 mL using brine solution and maintained at 37°C for 24 h under the light of incandescent lamps and surviving larvae were counted. Each experiment was conducted along with control (vehicle treated), at various concentrations of the test substance in each set that contained 6 tubes and the average results are reported (Table-2). The percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. LC_{50} values were obtained from the plot drawn: concentration (μg) *vs.* percentage inhibition. Podophyllotoxin was used as a standard.

Estimation of polyphenols: Polyphenol content in various extracts of green tea and black tea was estimated, using Folin-Ciocalteu (phenol) method[14], based

on reaction of polyphenols with folin-phenol reagent to yield blue colour complex which was measured using spectrophotometer at 760 nm.

RESULTS AND DISCUSSION

Aqueous extracts of green tea and black tea were found to scavenge the superoxide radicals (generated by photoreduction of riboflavin) and DPPH radicals. Aqueous extracts of green tea, however, showed higher antioxidant activity compared to black tea extracts. The antioxidant activity was enriched in ethyl acetate soluble portions of green tea as well as black tea extracts (Fig. 1). The antioxidant activity corroborates well with the polyphenol content of various fractions. The results are noted in Table-1.

TABLE-1
SUPEROXIDE AND DPPH RADICAL SCAVENGING ACTIVITIES*

S. No.	Test substances (Polyphenol content)	Superoxide			DPPH		
		D[a]	% Inh.[b]	IC_{50}	D[a]	% Inh.[b]	IC_{50}
1.	GT aq. ext. (40.67)	1	28.91	2.2	2	24.37	4.7
		2.5	54.21		5	54.26	
		5	73.99		10	81.75	
2.	GT EtOAc insol.	2.5	12.31	8.0	5	28.77	18.0
	(12.07)	5	32.27		10	37.94	
		10	51.37		25	60.25	
3.	GT EtOAc insol.	1	30.06	2.0	1	20.94	2.4
	(60.33)	2.5	58.59		2	44.00	
		5	78.27		5	90.38	
4.	BT aq. ext. (29.38)	2.5	24.20	6.1	5	33.35	9.2
		5	45.80		10	53.80	
		10	65.40		20	84.89	
5.	BT EtOAc insol.	5	31.30	12.0	5	15.96	20.0
	(25.13)	10	48.30		10	29.18	
		20	57.90		25	84.89	
6.	BT EtOAc insol.	2.5	43.60	4.1	2.5	28.52	4.4
	(44.67)	5	53.90		5	56.39	
		10	61.00		10	90.68	
7.	Vitamin C	50	17.67	150.0	2.5	43.46	2.5
		100	67.51		5	78.85	
		200	97.52		10	91.63	
8.	Vitamin E	100	22.12	312.5	1000	9.58	5500
		200	32.03		2500	23.87	
		400	62.96		5000	45.34	

*Values are mean of 3 tubes for each dose.
[a] Concentration in μg per mL.
[b] Percentage inhibition.

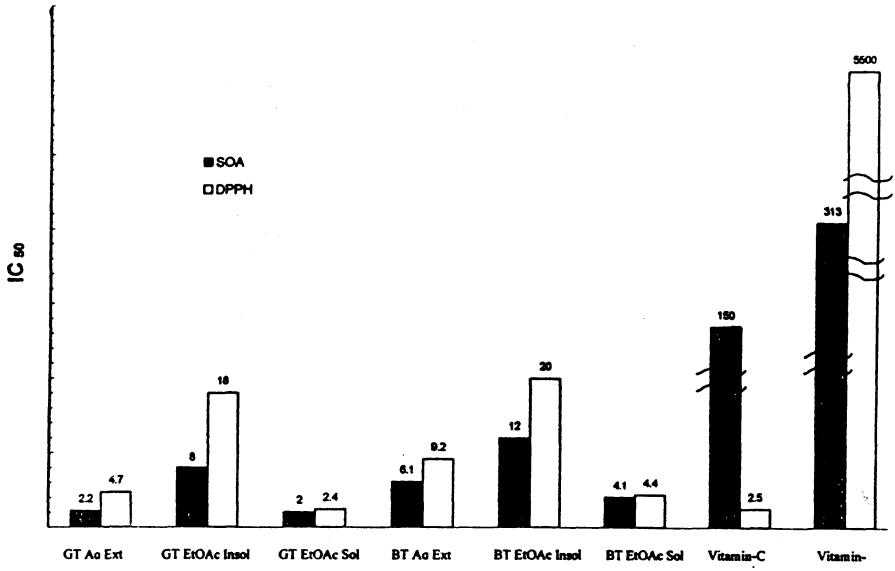

Fig. 1. Antioxidant activity

The green tea and black tea extracts showed low to moderate brine shrimp lethality and the results are presented in Table-2.

TABLE-2
BRINE SHRIMP LETHALITY DATA*

Test substance		Concentration of drug in µg/mL										
		0	25	50	100	150	200	250	300	400	500	LC_{50}
GT aq. ext.	MSL[b]	9.33	9.00	7.67	5.33	4.00	1.67	0.33	—	—	—	125
	% Lethality	C[a]	3.57	17.86	42.86	57.14	82.14	96.43	—	—	—	
GT EtOAc insol.	MSL[b]	8.00	—	—	6.67	—	5.67	—	5.00	4.00	3.00	400
	% Lethality	C[a]	—	—	16.67	—	29.17	—	37.5	50.00	62.50	
GT EtOAc sol.	MSL[b]	9.33	--	8.00	7.00	6.33	5.33	2.67	—	—	—	240
	% Lethality	C[a]	—	14.28	25.00	32.14	42.86	71.43	—	—	—	
BT aq. ext.	MSL[b]	9.00	8.00	7.33	6.33	4.67	2.33	0	—	—	—	154
	% Lethality	C[a]	11.11	18.52	29.63	48.15	74.07	100	—	—	—	
BT EtOAc sol.	MSL[b]	9.33	—	7.33	5.33	5.00	3.33	0.67	—	—	—	157.5
	% Lethality	C[a]	—	21.43	42.86	46.43	71.43	92.86	—	—	—	
Concentration		**0**	**0.1**	**0.25**	**0.50**	**1.00**	**2.5**	**5.0**				
Podophyllo-toxin	MSL[b]	8.33	8.00	6.50	6.00	5.60	4.80	1.00	—	—	—	2.75
	% Lethality	C[a]	4.00	22.00	28.00	32.80	42.00	94.00	—	—	—	

*Values are mean of 6 tubes for each dose.
[a] Considered as 0% lethality.
[b] Mean survival larvae.

Conclusion

The antioxidant activity of green tea is higher than black tea. The present study lends further support to the usage of green tea as a preferred beverage and nutraceutical.

ACKNOWLEDGEMENTS

The authors thank Sri G. Ganga Raju, Chairman, Laila group, Mr. G. Rama Raju, Director, Laila Impex for encouragement and for the sample of podophyllotoxin.

REFERENCES

1. T. Fleming (Ed.), PDR for Herbal Medicine, 1st Edn., Medical Economics Company, Montvale, New Jersey, USA, p. 710 (1999).
2. I.S. Bhatia, The Wealth of India: Raw Materials, CSIR, New Delhi, Vol. 3, p. 98 (1992).
3. D. Bagchi, A Keats Good Health Guide, Keats Publishing, Inc., Los Angeles, USA, p. 10 (1999).
4. M. Gill, Tea Cultivation to Consumption, Chapman & Hall, London, p. 570 (1992).
5. V. Marks, Tea Cultivation to Consumption, Chapman & Hall, London, p. 707 (1992).
6. B. Halliwell, *Lancet*, **344**, 721 (1994).
7. M. Ajitha and K. Rajnarayana, *Indian Drugs*, **38**, 545 (2001).
8. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, *Planta Med.*, **45**, 31 (I 982).
9. Sadhana Sathaye, A.R. Juvekar and R.D. Kulkarni, *Indian Drugs*, **38**, 569 (2001).
10. J.L. McLaughlin, C.J. Chang D.L. and Smith, Bench Top Bioassay for Bioactive Natural Products: An Update, Studies in Natural Product Chemistry, Vol. 9, Elsevier Science Publications, p. 383 (1991).
11. ———— , Simple Bench Top Bioassay for Discovcry of Plant Antitumor Compounds, American Chem. Soc. Symp., Ser. 534, p. 114 (1992).
12. J.M. McCord and I. Fridovich, *J. Biol. Chem.*, **244**, 6049 (1969).
13. J.I. Lamison, C. Ptitjean-Freytet and A. Carnet, *Pharma Acta Helv.*, **66**, 185 (1991).
14. V.L. Singleton, A. Joseph and J.R. Rossi, *Amer. J. Enology and Viticulture*, **16**, 144 (1999).

(*Received*: 10 December 2002; *Accepted*: 22 January 2003) AJC-2990