D-5643
[1-4]

RESEARCH ARTICLE Agricultural Science Digest

Phenolic Content and Characterization of Tea Shrub Bottom Leaves

Ajay T. Tandale, B.B. Nayak, K.A. Martin Xavier,
A.A. Devangre[1], Sandeep B. Gore, Amjad K. Balange

10.18805/ag.D-5643

ABSTRACT

Background: Tea polyphenols are extracted from top two - four young leaves and leaf buds of tea plant, while the bottom leaves of tea shrub are considered as an agricultural waste. Hence, an attempt has been made to utilise them as an alternate source of low-cost natural antioxidant.

Methods: Green tea shrub bottom leaves of *Camellia sinensis* Var. *assamica* /TV-23 were procured from Estate- Seni Mora Bagan, Karanga kamar, Hazarika Gaon, Tal. Karga Dist. Jorhat, Assam. Proximate analysis, total phenolic (Folin-Ciocalteau) and qualitative analysis (Agilent LC-MS system) for phenolic compounds including antioxidant properties was carried out using standard analytical methods.

Result: The results for proximate content were protein (14.43%), lipids (4.44%), total ash (4.94%), crude fibre (33.63%) and moisture (3.20%). A total phenolic content of dry green tea powder extracts prepared by using ethanol was 105.68 mg GAE/g dry tea powder. Total 12 different bioactive compounds were identified by HR-LCMS. The antioxidant properties were DPPH (89.82%), ABTS (74.71%), FRAP (1053.92 µ mol Trolox/gram dry tea powder) and metal chelating activity (17.51%). Based on the study we opine that; tea shrub bottom leaves can act as a promising source of low-cost tea polyphenols when extracted with ethanol as a solvent for value added fish product shelf life extension.

Key words: Antioxidants, Green tea extract, HR-LCMS, Tea polyphenols, Tea shrub bottom leaves, Total phenolic content.

INTRODUCTION

Preparing value added products by utilising fish meat is a viable option to improve the livelihood of people. However, it is widely known that fishes are highly perishable food commodities,spoil faster andproper measures to delay spoilage needs to be undertaken for extending shelf-life (Feng *et.al*., 2012). Natural extracts of plant origin having antioxidants and antimicrobial compounds are used to preserve meat and are well known to retard oxidation, improve shelf life and stability of fish products (Gai *et al*, 2014).

Around 6.37 lakh hectares of land is under tea cultivation producing 1,283 million kg tea during 2020-21 which makes India the second-largest global tea producer. Assam, West Bengal, Tamil Nadu and Kerala are the major tea-producing states. The health benefits ascribed to the high content of bioactive ingredients like catechins, flavanols and flavones are also present in tea leaves (Khan and Mukhtar 2007).

Polyphenols have been reported to possess antioxidant, antiviral and anti-inflammatory activities; modulate detoxification enzymes; stimulate immune function and decrease platelet aggregation (Siripatrawan and Noipha, 2012). However, black and green tea is made from the top two-four young tea leaves and leaf buds of tea plant and old tea leaves are considered an agricultural waste (Karaosmanoglu and Kilmartin, 2015). Hence, an attempt has been made in the present study to utilise the green tea shrub bottom leaves of *Camellia sinensis* as an alternate source of low-cost natural antioxidant for value addition in fish meat.

Department of Post-Harvest Technology, ICAR-Central Institute of Fisheries Education, Versova, Mumbai-400 061, Maharashtra, India.

[1]Department of Livestock Product Technology, College of Veterinary and Animal Sciences, Udgir, Latur-413 517, Maharashtra, India.

Corresponding Author: Amjad K. Balange, Department of Post-Harvest Technology, ICAR-Central Institute of Fisheries Education, Versova, Mumbai-400 061, Maharashtra, India.
Email: amjadbalange@cife.edu.in

How to cite this article: Tandale, A.T., Nayak, B.B., Xavier, K.A.M., Devangre, A.A., Gore, S.B. and Balange, A.K. (2023). Phenolic Content and Characterization of Tea Shrub Bottom Leaves. Agricultural Science Digest. DOI: 10.18805/ag.D-5643.

Submitted: 25-07-2022 **Accepted:** 09-12-2022 **Online:** 12-01-2023

MATERIALS AND METHODS

The present experiment was conducted during 2018-19 at the Post-Harvest Technology Laboratory of Indian Council of Agricultural Research (ICAR) -Central Institute of Fisheries Education, Mumbai, India. All the chemicals and reagents used in the present study were obtained from Merck (Mumbai), M/S. SD-Fine Chemicals (Mumbai) and M/S. Loba

(Mumbai) and were of analytical grade (AR) or guaranteed grade (GR). The glassware manufactured by Borosil and Schott Duran were used during the study.

Preparation of green tea shrub bottom leaves powder

During the present study green tea shrub bottom leaves of *Camellia sinensis* Var. *assamica* /TV-23 were procured from Estate- Seni Mora Bagan, Karanga kamar, Hazarika Gaon, Tal. Karga Dist. Jorhat, Assam. The collected green tea leaves were air dried for 4 days and then dried in hot air dryer at 60°C for 5 hours and finally grinded in mixer grinded and sieved through 0.5 mm sieve for getting fine powder and used as a source of green tea powder.

Proximate composition analysis of green tea shrub bottom leaves powder

The proximate analysis such as moisture total ash, crude protein, ether extract, crude fiber, nitrogen free extract and dry matter was carried out by following prescribed methods by AOAC (1995).

Quantitative and qualitative analysis

The crude extract from black tea powder was prepared using ethanol as solvents following method with slight modifications chew *et al.* 2008). The extract was stored in air tight container and kept in refrigerator until further analysis was carried out. The total phenolics of ethanolic crude extract were determined by a spectrophotometer method of Folin-Ciocalteau reagent (Lin and Tang, 2007). Further, the tea extract was analysed qualitatively for phenolic compounds by following the method of Lakshmi (2017) at SAIF, IIT Mumbai.

Determination of antioxidant activity

Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of crude green tea powder extract was measured according to the method of Shimada *et.al.* (1992). The FRAP (Ferric Reducing Antioxidant Power) assay of crude black tea powder extract was measured according to the method of Thaipong *et al.* (2006). The results were expressed as µ mol. of Trolox equivalent / g dry tea powder. The ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] activity of crude black tea powder extract was measured according to the method of Sumczynski *et al.* (2015) with some modification. Absorbance of the mixture was measured by using a spectrophotometer at 734 nm. Further the chelation of ferrous ions of crude black tea powder extract was estimated by method of Dinis *et al.* (1994).

Statistical analysis

Data were subjected to one-way analysis of Variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel and Torrie, 1980) using the Statistical Package for Social Science (SPSS 8.0 for windows, SPPS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Proximate composition

The results of the proximate composition of green tea leaves powder are presented in Table 1 along with its antioxidant properties. The data indicated that, the moisture content in green tea powder was found to be 3.20± 0.10%. The results of present findings are in close conformity with findings reported by Khanum *et al.*, (2017) who stated that the mean moisture content in tea powder of Northern and Southern India teas ranged from between 3.5 to 6.0%. As per the Indian standards for Green tea, total ash and maximum crude fibre content should be 4-8% and 16.5%, respectively (Anonymous, 2003). In the present study ash obtained was 4.94% which is line with the Indian standards.Whereas, crude fibre obtained in the present study was 33.63%. Lipids constitute about 2-3% by weight of unprocessed tea (Stagg and Millin,1975). In the present the lipid content was found tobe4.44± 0.22%. The high content of crude fibre and lipids, in present study may be attributed to utilisation of lower tea shrub leaves.

Protein content on dry weight basis from green teawas 14.43±0.15% which is in accordance with findings of Angga *et. al.* (2018) who reported the range of 15-20% in green tea powder from Pakistan. The slight lower range of protein content may be attributed to higher lipid content and selection of lower tea shrub leaves.

Over all the proximate composition of bottom shrub tea leaves used in the study is in lines with other studies regarding moisture and ash content in green tea leaves but shows lower protein, high fat and fibre content which attributed the utilisation of lower tea shrub leaves.

Quantitative and qualitative analysis

The total phenolic content (TPC) depends on many factors. Highest levels were present in the bud and first leaf and lowest levels in the internodes (Khanum *et al.,* 2017). The results of TPC in the present study are 105.68 mg GAE /gm

Table 1: Proximate composition and antioxidant assay of green tea powder.

Particulars	Green tea powder
Moisture (%)	3.20±0.10
Crude protein (CP) (%)	14.43±0.15
Ether extract/Lipid (%)	4.44±0.22
Total ash (%)	4.94±0.05
Crude fiber (%)	33.63±0.10
Nitrogen free extract (NFE) (%)	42.56±0.22
Dry matter (%)	96.80±0.10
DPPH (%)	89.82±0.13
FRAP (µ mol Trolox/gram dry tea powder)	1053.92±0.52
ABTS (%)	74.71±0.20
Metal chelating activity (%)	17.51±0.05

Note: Data are expressed as the mean ± SD (n=3).

dry tea powder. (Unachukwu *et. al.* 2010) estimated TPC from 19 samples of loose leafs of green tea using water as a solvent in range of 1.17 to 18.59 mg GAE/g dry tea.

Khokar and Magnusdottir (2002) estimated TPC by using ethanol solvent from three brands of green tea in supermarkets of United Kingdom in the range of 65.8 mg GAE/g to 106.2 mg GAE/g. The results of present experiment are parallel with earlier results reported by various workers.

Total of seven bioactive compounds were extracted from green tea using ethanol as solvent *viz*., Alkaloids- (Caffeine), (Theophylline) and Flavonoid derivatives-(Cosmosiin), Catechin derivatives-(Epigallocatechin Gallate and Epicatechin gallate) and Flavanol-(Fisetin) and Derivative of Glucuronic acid-(Ramipril Glucuronide).

Majority of the total polyphenol content of green tea is attributed to catechins (85%) and the four major catechins are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (Karaosmanoglu and Kilmartin, 2015). EGCG and ECG are quality markers of green tea and are known as powerful antioxidants in tea (Esmaeelpanah *et al*., 2018). Further, caffeine which is responsible for their higher antioxidant activity of tea is present in tea and absent in most herbal teas. Caffeine is also less sensitive to heat as well as does not undergo considerable reduction during processing and its stimulant properties persist in contrast to catechins (Astill *et al.,* 2001). Cosmosiin, also known as Apigenin representsa small fraction of the polyphenols present in tea is known for moderate anti-oxidant activity. Also several studies indicate that Fisetin is a promising novel antioxidant (Khan *et al.,* 2013).

Therefore, bottom tea shrub leaves usually treated as an agricultural wasteis found to have high TPC, along with EGCG, ECG, caffeine, Cosmosiin and Fisetin which can act as low cost natural polyphenol source for value addition.

Antioxidant properties

The antioxidant properties of bottom tea shrub leaves green tea extract were determined for DDPH, FRAP, ABTS and Metal chelating activity. The results for antioxidant properties were DPPH (89.82%), ABTS (74.71%), FRAP (1053.92 µ mol Trolox/gram dry tea powder) and Metal chelating activity (17.51%). Nadiahand Uthumporn (2015) reported 89.14% DPPH activity of green tea extracts prepared using 50% ethanol. They also further stated that higher the DPPH inhibition indicates higher antioxidant activity in tea (Nadiah and Uthumporn, 2015). The DPPH activity in branded green tea of India is reported to be 92.84% (Pal *et al.,* 2012). Further the green tea provides approximately 1300 µmol of Trolox equivalents per gram of dried tea leaves as per Chandra and de Mejia (2004). Shalaby *et al.* (2016) reported 75% to 95.8% ABTS activityfrom green tea using 50 µg/ml concentration and 100 µg/ml concentration respectively. The metal chelating activity of green tea extract prepared with ethanol based extraction is reported to be 23.21% and ranged from 22.45% to 33.50% for 11 different tea types (Oh *et al.,* 2013). The results of present study with respect to DDPH, FRAP, ABTS and Metal chelating activity are in line with these studies except showing a slight lower value of FRAP which may be due to utilisation of bottom shrub tea leaves.

CONCLUSION

Based on the findings of this preliminary study it is opined that, tea shrub bottom leaves, which can act as a promising, alternate, natural and safe additive source of low cost tea polyphenols for incorporation as an antioxidant and antimicrobial agent invalue added fish product for extension of shelf life.

Conflict of interest: None.

REFERENCES

Angga, W.A., Rizal, Y., Mahata, M.E., Yuniza, A. and Mayerni, R. (2018). Potential of waste tea leaves (*camellia sinensis*) in west Sumatra to be processed into poultry feed. Pakistan Journal of Nutrition. 17: 287-293.

Anonymous (2003). IS 15344: Green tea [fad 6: stimulant foods]. Indian Standard Green Tea- Specification. ICS 67.140.10, Bureau of Indian Standards Manak Bhavan, 9 Bahadur Sah Zafar Marg, New Delhi, pp.1-18.

AOAC. (1995). Official Methods of Analysis, Association of Official Analytical Chemists, 16th Ed., Washington DC, USA.

Astill, C., Birch, M.R., Dacombe, C., Humphrey, P.G. and Martin, P.T., (2001). Factors affecting the caffeine and polyphenol contents of black and green tea infusions. J. Agric. Food Chem. 49(11): 5340-5347. DOI:10.1021/jf010759+.

Chandra, S.M. and de Mejia, E.G. (2004). Polyphenolic compounds, antioxidant capacity and quinone reductase activity of an aqueous extract of Ardisiacompressa in comparison to mate (*Ilex paraguariensis*) and green (*Camellia sinensis*) teas. J Agric. Food Chem. 52(11): 3583-3589.

Chew, Y.L., Lim, Y.Y., Omar, M. and Khoo, K.S. (2008). Antioxidant activity of three edible sea weeds from two areas in South East Asia. Science Direct LWT- Food Science and Technology. 41(6): 1067-1072.

Dinis, T.C.P., Madeira, V.M.C. and Almeida, M.L.M. (1994). Action of phenolic derivates (Acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169.

Esmaeelpanah, E., Razavi, B.M., Hasani F.V. and Hosseinzadeh, H. (2018). Evaluation of epigallocatechin gallate and epicatechin gallate effects on acrylamide-induced neurotoxicity in rats andcytotoxicity in PC 12 cells. Drug Chem. Toxicol. 41: 441-448. DOI:10.1080/01480545.2017.1381108.

Feng, L., Jiang, T., Wang, Y. and Li, J. (2012). Effects of tea polyphenol coating combined with ozone water washing on the storage quality of black sea bream (*Sparus macrocephalus*), Food Chem. 135: 2915-2921.

Gai, F., Gasco, L., Ortoffi, M., Gonzales-Rodriguez, A. and Parisi, G. (2014). Effects of green tea natural extract on quality parameters and lipid oxidation during storage of tench (*Tinca tinca*) fillets. Journal of Applied Ichthyology, J. Appl. Ichthyol. 30(Suppl. 1): 4-71.

Karaosmanoglu, H. and Kilmartin, P.A. (2015). Tea extracts as antioxidants for food preservation. Handbook of Antioxidants for Food Preservation. Pp: 219-233.

Khokhar, S. and Magnusdottir, S.G.M. (2002). Total phenol, catechin and caffeine contents of teas commonly consumed in the united kingdom. Journal of Agricultural and Food Chemistry. 50: 565-570.

Khan, N., Syed, D.N., Ahmad, N., Mukhtar, H. (2013). Fisetin: A dietary antioxidant for health promotion. Antioxid. Redox Signal. 19: 151-162. DOI:10.1089/ars.2012.4901.

Khan, N. and Mukhtar, H. (2007). Tea polyphenols for health promotion. Life Sci. 81(7): 519-33. DOI: 10.1016/j.lfs.2007. 06.011.

Khanum, H., Faiza, S., Sulochanamma, G. and Borse, B.B. (2017). Quality, antioxidant activity and composition of Indian black teas. J. Food Sci. Technol. 54(5): 1266-1272.

Lakshmi, S. (2017). PG thesis on Use of seaweed extract as a natural preservative for chill stored fish. In PG Thesis ICAR-CIFE, Mumbai: 1-88.

Lin, J.Y. and Tang, C.Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Science Direct, Food Chemistry. 101(1): 140-147.

Nadiah N.I. and Uthumporn, U. (2015). Determination of phenolic and antioxidant properties in tea and spent tea under various extraction method and determination of catechins, caffeine and gallic acid by HPLC. Int. J. Adv. Sci. Eng. Inf. Technol. 5: 158-164. DOI:10.18517/ijaseit.5.3.520.

Oh, J., Jo, H., Cho, A.R., Kim, S.J. and Han, J. (2013). Antioxidant and antimicrobial activities of variousleafy herbal teas. Food Control. 31(2): 403-409. DOI:10.1016/j.foodcont. 2012.10.

Pal, S., Ghosh, D., Saha, C., Chakrabarti, A.K., Datta, S.C. and Dey, S.K. (2012). Total polyphenol content, antioxidant activity and lipid peroxidation inhibition efficacy of branded tea (*Camellia sinensis*) available in India.International Journal of Tea Science. Vol. 8 No. 3: 13-20. DOI: https://doi.org/10.20425/ijts.v8i3.4720.

Shalaby, E.A., Mahmoud, G.I. and Shanab, S.M.M. (2016). Suggested mechanism for the effect of sweeteners on radical scavenging activity of phenolic compounds in black and green tea. Frontiers in Life Science. 9(4): 241-251. DOI: 10.1080/21553769.2016.1233909.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. Journal of Agriculture Food Chem. 40: 45-948.

Siripatrawan, U. and Noipha, S. (2012). Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. Food Hydrocolloids. 27(1): 102-108. DOI:10.1016/j.foodhyd.2011.08.011.

Stagg, G.V. and Millin, D.J. (1975).The nutritional and therapeutic value of tea-A review. Journal of the Science of Food and Agriculture. 26(10): 1439-1459. DOI: 10.1002/jsfa. 2740261002.

Steel, R.G.D. and Torrie, J.H. (1980). Duncan's New Multiple Range Test. In: Principles and Procedures of Statistics: [C. Napier and J.W. Maisel (Eds.)]. 187-188.

Sumczynski, D., Bubelova, Z., Sneyd, J., Erb-Weber, S. and Mlcek, J. (2015). Total phenolics, flavonoids, antioxidant activity, crude fibre and digestibility in non-traditional wheat flakes and muesli. Journal of Food Chemistry. 174: pp- 319- 325.

Thaipong, K., Boonprakob, U.,Crosby, K., Cisneros-Zevallos, L. and Byrne, D.H. (2006). Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis. 19(6): 69-675.

Unachukwu, U.J., Ahmed, S., Kavalier, A., Lyles, J.T. and Kennelly, E.J. (2010). White and green teas (*Camellia sinensis var. sinensis*): variation in phenolic, ethylxanthine and antioxidant profiles. Journal of Food Science. 75(6): 541-548. DOI: 10.1111/j.1750-3841.2010.01705.x.