Journal of Advanced Research in Biochemistry and Pharmacology
Volume 2, Issue 2 - 2019, Pg. No. 14-19
Peer Reviewed Journal

Research Article

Evaluation of Secondary Metabolites and Proximate Composition in Different Varieties of Green Tea

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How to cite this article:
Bashir A, Waheed A, Hamid FS et al. Evaluation of Secondary Metabolites and Proximate Composition in Different Varieties of Green Tea. *J Adv Res Biochem Pharma* 2019; 2(2): 14-19.

Date of Submission: 2019-05-29
Date of Acceptance: 2019-06-20

A B S T R A C T

Leaves of *Camellia sinensis* are composed of a variety of secondary metabolites play a vital role in human nutrition and health. In this study three different varieties of processed green tea includes P3, P5 and P9 were collected from National Tea and High Value Crops Research Institute (NTHRI), Shinkiari, Mansehra, Pakistan for proximate composition analysis and secondary metabolites analysis. Standard methods were carried out to evaluate proximate composition analysis of crude fat, crude fiber, ash content and moisture content in all tea samples. Presence of secondary metabolites in aquaeous and ethanolic tea leaves extracts was recognized by its colour intensity using standard chemical tests. Composition of moisture content, ash content, crude fiber and crude fat in tea samples were range between 3.2% to 6.9%, 6% to 5.4%, 10.4% to 15.6%, 3.5% to 10.1% respectively. Secondary metabolites like alkaloids, saponins and flavonoids were found positive in aqueous extract but absent in ethanolic extract of all samples while phlobatannin and carotenoids were found absent in both tea extracts.

Keywords: *Camellia Sinensis,* Phlobatannin, Terpenoids, Neuroprotective Properties

Introduction

Camellia sinensis is an evergreen shrub and non alcoholic beverage, known to man for long time. Leaves of this plant are used for tea production. 20% production of Camellia leaves appears as green tea, which is primarily consumed in Asia, United States, various parts of North Africa and also in Europe. The correlation between tea utilization, particularly green tea and human health has long been valued (Weisburger *et al.,* 2000) (Stato and Miyata, 2000) in 1985-86, a tea research station was established in Shinkiari, District Mansehra under the direction of the Pakistan Agriculture Research Council (PARC) (NTRI, 2012).

Tea is the infused leaves extract of *C. sinensis* and one of the most consumable drink worldwide (Xiao *et al.,* 2008). Statistics reveal that the average consumption of tea around the world is 4 fluid ounces per day per capita (Zhu *et al.,* 2006). While per capita utilisation of tea in Pakistan is one kilogram after United Kingdom so the second largest tea

importing country is Pakistan that imports unprocessed and processed tea from other countries (Latif *et al.,* 2008).

Chemically tea is composed of caffeine, polysaccharide, polyphenols, the aflavin, the arubigin, amino acids, sugar, alkaloids, minerals, volatile acids, trace elements and the essential oil. Overall quality of tea such as colour, aroma and flavour is due to all these factors (Monobe *et al.,* 2008; Wei *et al.,* 2010; Xiong *et al.,* 2012).

Secondary metabolites in tea plant are categorized by three most important groups which are phenolic compounds, terpenoids and nitrogenous compounds. Polyphenol content mainly flavanols in green tea which be the 30% of fresh leaf dry weight have health-promoting effects (McKay and Blumberg, 2002; Naghma and Husan, 2007).

P3, P5 and P9 are tea clones introduced by Unilever Pakistan and now cultivated in PARC NTHRI as well.

Tea has efficacy in preventing cardiovascular diseases, anti-cancer property (McKay and Blumberg, 2002); (Kavanagh *et al*., 2001; Sueoka *et al*., 2001), anti-inflammatory (Dona *et al*., 2003), anti-oxidative (Osada *et al*., 2001), antibacterial (Roccaro *et al*., 2004), antiviral (Weber *et al.,*2003), anti-angiogenic (Sartippour *et al*.,2002), anti-arthritic (Haqqi *et al*., 1999), neuroprotective properties (Weinreb *et al*.,2004). Inspite of all these health benefits affects it may cause other serious health concerns such as tea catechins have an effect on iron absorption, predominantly in groups at menace on deficiency of iron (Samman*et al*., 2001; Nelson and Poulter, 2004). Moreover, the level of zinc would be decrease and amount of manganese increases correspondingly by over intake of green tea (Deng *et al*., 1998); therefore green tea catechins possessed the ability to affect on absorption and metabolism of ions due to flavonoids interaction with several ions of metals (Mira *et al*., 2001).

Keeping in view, the physiological importance of minerals in green tea, the present study was design to analyze secondary metabolites, proximate composition of different varieties of green tea (P_3, P_5, and P9).

Materials and Methods

All the research work pertaining to this investigation was carried out in PARC NTHRI, Shinkiari and Comsats University of information and technology Abbottabad, Pakistan.

Figure 1.Tea varieties P3, P5 and P9 collected from PARC NTHRI, Shinkiari

Sample Collection and Preparation

Processed leaves of green tea varieties P3, P5, P9 were collected from PARC NTHRI.

All tea samples were powdered and placed in desiccators to prevent gaining moisture. Powdered samples were used to prepare aqueous and ethanolic extract by soaking them in 50 ml solvent for 24 hours then filtered through a filter paper (125 mm). These both extracts were used for the analysis of secondary metabolites by standard chemical tests. Presence of secondary metabolite was confirmed by the intensity of colour change.

Test for Tannin

Tannin was determined by the ferric chloride test which was described by (Harbone, 1973) the same was reported by (Osagie, 2011). 2ml of the aqueous and ethanolic extracts were taken in test tubes then 3ml of distilled water was added to each test tube, shaken well on shaker apparatus to make homogeneous mixture following which two drops of dil. Ferric chloride ($FeCl_3$) were added. Dark green precipitate was appeared which was the indication of tannin.

Test for Saponins

Saponins presence was determined by the method of (Harbone, 1973) now reported by (Osagie, 2011). It is also called the Froth test because of the froth formation. In test tubes 2 ml aqueous and ethanolic extract were added along with 6 ml of distilled water. The mixture was shaken very well and froth was formed indicates the presence of saponins.

Test for Alkaloids

Method described by (Okwu, 2005) was used for the detection of alkaloids content in each sample of tea. 5ml of 2% HCl was added in 2ml of both ethanolic and aqueousextracts then heated gently on steam bath and filtered by Whatman filter paper. After this 0.5 ml of Wagner's reagent was added in 1 ml of each filtrate. A reddish brown precipitate was formed which indicated the presence of alkaloids.

Test for Flavonoids

Acid-alkaline test which was described by (Osagie, 2011) was used for the determination of flavonoids presence in tea samples. For this, few drops of concentrated Ammonia were added in 2 ml of ethanolic and aqueous extracts. This gave yellow colour, which confirmed the presence of flavonoids.

Test for Phlobatannins

2% aqueous HCl drop by drop was added in 1ml of each extract of tea sample and boiled for few minutes. Red precipitates appeared were the sign of the presence of phlobatannins (Sofowora *et al*., 1993).

Test for Phenol

$FeCl_3$ Test: Few drops of 10% aqueous $FeCl_3$ was added in 2 ml of both aqueous and ethanolic extract blue green colour emerged which was the sign of phenol (Sofowora *et al*., 1993).

Test for Glycosides

$FeCl_3$ Test: 5ml of conc. H_2SO_4 was treated with 2 ml of ethanolic and aqueous extracts then boiled for 15 min using water bath. After that mixtures were cooled and 20% KOH used to neutralize the mixtures. Added few drops of $FeCl_3$ into both mixtures and green to black precipitates were appeared (Sofowora *et al.*, 1993).

Test for Steroids

H_2SO_4 Test: Took 2 ml of ethanolic and aqeuous extracts in test tubes, added 6 drops of concentrated H_2SO_4 cautiously from the side wall of the test tube treated with was added. Red colouration showed the presence of steroids (Idu and Igeleke, 2012).

Test for Terpenoids

Salkowaki Test: 1 ml of both aqueous and ethanolic extract was treated with 2 ml of chloroform and added 3 ml of concentrated H_2SO_4. At interface reddish brown colour appeared (Sofowora *et al.*, 1993).

Test for Carotenoids

10 ml of chloroform was added in 2 ml of each aqueous and ethanol extracts in test tubes and shaken very well. Then mixture was filtered by using filter paper and treated with 85% sulphuric acid. Presence of carotenoids was recognized by the appearance of blue green colour (Sofowora *et al.*, 1993).

Sample preparation for Proximate Analysis

Leaves of different varieties of processed green tea i.e., p3, p5, p9 samples were taken then powdered and used for proximate analysis.

Proximate Composition

The samples of green tea were investigated for proximate analysis i. e. crude fiber and crude fat contents, moisture and ash contents. These determinations were performed in triplicate in accordance with process as illustrated by AACC (2000).

Moisture Determination

Moisture content of different green varieties was determined by using an official method 44-01 of AACC (2000). First the weight of the Petri dishes was noted then added 3g of each tea sample in them, these dished was placed in an oven at 105^0c and set the time duration 24 hours. After this, it was transferred from oven to desiccators for 30 min. The petri dishes with the sample were weighted again and percent moisture was calculated by using the following formula:

$$\% \text{ moisture} = \frac{weight\ loss}{weight of sample} \times 100$$

Ash Determination

Estimation of ash content was done accordance with the process demonstrated by AACC (2000) method 08-01. Taken weighted crucibles with 2 g of each tea sample and ignited through an oxidizing flam for few minutes then placed in a furnace at 550°C for 6 h. The crucibles then shifted to desiccators for coolness, reweight and determined.

$$\% \text{ ash} = \frac{wt.\ of ash}{wt.\ of sample} \text{ x } 100$$

Crude Fat Determination

Soxhlet apparatus was used for the analysis of crude fat accordance with the AACC (2000) official method 30-10. 2 g of moisture free sample of tea was taken and wrapped in filter paper then this wrapped sample was placed in thimble to extraction tube of the soxhlet apparatus. Pre- weighted, clean and dried soxhlet flask was taken and added 75 ml solvent (pet. Ether 40-60°C). The flask was connected with extraction tube. The apparatus was adjusted followed by water flow and heating system. After 7-8 siphoning the flask was removed and ether was evaporated on water bath. The oil collecting flask was transferred to an oven at 105°C, until constant weight was obtained. The flask was cooled in desiccator, and reweighed Percent crude fat was determined as:

$$\% \text{ crude fate} = \frac{wt.\ of etherextract}{wt.\ of sample} \text{ x } 100$$

Crude Fiber Determination

Crude fiber was determined according to the procedure described by AACC (2000) method 32-10 after two specific conditions which followed as:

Acid Digestion

Two gram powdered sample (fat free) was transferred to 250 ml beaker, containing 200 ml of 1.25% H_2SO_4 heated on a stream bath at specific temperature 95^0C and time duration was approximately 2 h and then filtered by using linen cloth. To make the acid free washed the residue with hot water. The residue was transferred to another beaker for alkali digestion.

Alkali Digestion

The acid digested residue shifted in to an another beaker containing 200 ml of 1.25% NaOH was again digested for 2 h on steam bath and filtered through Gooch crucible. To make alkali free these residues washed with 25 ml ether, hot water and 50 ml alcohol. These residues were placed in an oven at 130 ^{0}C for 3 hours then transferred into desiccators

and weighted. Subsequent to this the residues was ignited at 550^{0}C for1 hour then cooled, reweighted and calculated the content of crude fiber.

$$\% \text{ crude fiber} = \frac{weightlossonignition}{weightofsample} \text{ x } 100$$

Statistical Analysis

Experimentation was conducted in triplicate and treatments of tea sample were examined by using analysis of variance under Completely Randomized Design (CRD) while mean values of the treatments compared by Least Significant. Difference (LSD) at 5%level of the probability ($p \leq 0.05$).

Result and Discussion

Secondary Metabolites

Different green tea varieties including P3, P5 and P9 were analysed for its secondary metabolites in two different solvent extracts i.e., distilled water extract and ethanolic extract. Investigation of these extracts showed the presence of tannins, phenols and steroids in both aqueous and ethanolic extracts. Saponins, alkaloids and flavonoids were found in aqueous extracts of all tea varieties but were absent in ethanolic extracts. Absence of these chemical constituents in ethanolic extracts may be hard to soluble in ethanol. Flavonoids are polar compounds, so are easily soluble in polar solvent; flavonoid polarity will grow in the presence of sugar bound in the form of glycosides which are more soluble in water (Hanani, 2015). Carotenoids and Phlobatannins were absent in both aqueous and ethanolic extracts in all tea varieties. Terpenoids were present in both aqueous and ethanolic extracts of P3 and P9 while they were absent in ethanolic extract of P5. Glycosides were present in both aqueous and ethanolic extracts of P3 and P9 while they were absent in aqueous extract of P5. The phytochemical screening revealed that the leaves of these tea plants are rich in tannins, phenol, steroid and terpenoids (Table 1). Secondary metabolites have the medicinal and physiological activity (Edeogal *et al.,* 2005). The phenolic compounds are considered to be one of the main group of plant metabolites (Singh et al., 2007). They exhibit the biological properties including the improvement of endothelial function, anti-apoptosis, anti-carcinogen, anti-aging, cardiac safety and anti-inflammation property (Han *et al.*, 2007). Previous research revealed that the antioxidant activity of herbs due to phenolic compounds (Berger 2001). Steroids play a vital role in hormonal balance especially in sex hormones and also have antiseptic properties (Okwu, 2001; Raquel, 2007). Flavonoids are an essential plant metabolites have function against cancer and heart disease (Noroozi *et al*., 1998). Inhibitory effect of saponins on irritation observed by the previous study (Just *et al*., 1998) while cholesterol binding and hemolytic properties of saponins are also substantial (Soipo *et al*., 2000). Tannins have antiseptics, antimicrobial, astringents and antioxidant activity which including anti-mutagenic and anti-carcinogenic property. Regardless of the fact that the higher amount of tannins be the reason of protein precipitation, enzyme inhibition (Chung *et al*., 1998).

Table 1.Tea varieties P3, P5 and P9 collected from PARC NTHRI, Shinkiari

Secondary Metabolites	Extract	P3	P5	P9
Tannins	Aqueous	+	+	+
	Ethanolic	+	+	+
Saponins	Aqueous	+	+	+
	Ethanolic	-	-	-
Alkaloids	Aqueous	+	+	+
	Ethanolic	-	-	-
Flavonoides	Aqueous	+	+	+
	Ethanolic	-	-	-
Phenol	Aqueous	+	+	+
	Ethanolic	+	+	+
Glycosides	Aqueous	+	-	+
	Ethanolic	+	+	+
Phlobatannins	Aqueous	-	-	-
	Ethanolic	-	-	-
Steroids	Aqueous	+	+	+
	Ethanolic	+	+	+
Terpenoids	Aqueous	+	+	+
	Ethanolic	+	-	+
Reducing Sugars	Aqueous	+	+	+
	Ethanolic	+	+	+
Gums and Mucilages	Aqueous	+	-	+
	Ethanolic	+	+	+
Carotenoids	Aqueous	-	-	-
	Ethanolic	-	-	-
Phytosterols	Aqueous	+	+	+
	Ethanolic	+	+	+

+ = positive/ present - = negative/ absent

Proximate Composition

Moisture Content

Moisture content in different varieties of green ranged between 3.2% to 6.9%.These results approved by the early findings of Iqbal (2002). Moisture content observed in P3 5.7%, P5 6.9% and in P9 3.2%. This indicated that P5 contained comparatively higher moisture content than P9 and P3. Moisture content of the tea based upon nature of

the tea and their drying time (Kurma *et al.,* 2005). In green tea higher moisture content may be due to prohibiting of fermentation process. Polyphenols of the tea eradicated during this process and as a result moisture content retained (Yao *et al*., 2006).

Ash Content

Ash content ranged from 6% to 5.4% different varieties of green tea. The present outcomes are the same to the previous results accounted by Iqbal (2002). In P3 sample the value of ash content observed 5.4% in P5 it was 6% and in P9 5.3%. This analysis showed that P5 contained higher amount of ash content as compared to P3 and P9. Ash content of tea is also a significant factor. Less moisture content is the reason of higher ash content in tea while extracted raw material used for the production of tea is the main cause less ash content in tea (Ismail *et al.,* 2000; Rehman *et al.,* 2002). Higher content of ash indicated that tea can serve as good sources of minerals reported by (Dawodu *et al*., 2013).

Crude fat

Fat analysis showed that fat content ranged between 3.5% to 10.1% in different varieties of green tea. Crude fat content in P3, P5 and P9 was 6.2%, 10.1%, 3.5% respectively. Highest amount of crude fat observed in P5 than P3 and P9. The highest amount of fat content observed in green tea might be due to lack of fermentation during the green tea processing. These results are in line to that results which reported by (Rehman *et al.,* 2002).

Crude Fiber

Crude fiber varied from 10.4% to 15.6% in different varieties of green tea which was supported by the findings of Iqbal (2002).Crude Fiber content in tea is also a central quality control factor. The concentration of crude fiber in P3 sample was 10.4% while in P5 it was 15.6% and in P9 12.6%. So it found that quantity of crude fiber was higher in P5 sample rather than P3 and P9. Higher fiber Content of tea may be by using the stems like impurities during its processing while low fiber content in tea samples due to use of younger leaves of tea plant. Moreover, the process of curling, tearing, and crushing also destroyed the structure of tealeaf and thus fiber content might be effected (Venkatesan *et al.,* 2006).

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