SHORT COMMUNICATION

Phytochemical and manufacturing profile of a sample of green tea formally sold in Lima, Peru

Enrique Moncada-Mapelli [1,2,3,b]; Kristel Yañez-Agapito [1,2,3,b]; Brenda Milla-Huerta [1,2,3,c]; Stephany Rojas-Monge [1,2,3,b]; Kiomi Cerda-Yupanqui [1,2,3,b]; Alberto Salazar-Granara* [1,2,4,5a]

ABSTRACT

Green tea, derived from the *Camellia sinensis* plant, has been prized throughout history for its potential medicinal properties. It has been shown to contain polyphenols, such as flavones and flavonoids, which possess antioxidant properties and may benefit cardiovascular health and protect against neurological diseases. Chemical compounds in green tea are identified using qualitative and quantitative techniques. This descriptive and comparative study analyzed three green tea samples collected at a licensed shopping center in Lima, Peru. A qualitative method called phytochemical screening was used, which allowed identifying the presence of secondary metabolites such as flavonoids, alkaloids, phenols and tannins. Likewise, the results of the present research were compared to the results of green tea samples collected in Cuzco (Peru) and Kano (Nigeria), referenced by previous studies using the same phytochemical screening method. The data are presented in descriptive tables. The main findings show a heterogeneous presence of tannins among the samples collected in Lima, and compared to the reference samples from Cuzco and Kano, possibly lower concentration of flavonoids and phenols. Regarding manufacturing, it was found that two samples collected in Lima did not comply with the current regulations in the items of scientific name of the product, medicinal use of the product, and secondary metabolite reference. This study demonstrates heterogeneity in the phytochemical and manufacturing profile of the green tea samples collected in Lima, a situation that could be reflected in aspects of safety and efficacy for consumers. Therefore, further research on green tea for human consumption is needed to broadly understand the benefits versus risks due to its impact on human health.

Keywords: Green tea; Phytochemicals; Manufactured Materials; Camellia sinensis (Source: MeSH NLM).

INTRODUCTION

Green tea (*Camellia sinensis*) is a beverage that has a deep-rooted history in human culture. It has been traditionally attributed medicinal properties, and in recent years, interest in its health benefits has increased, accompanied by numerous studies. The processed leaves of *Camellia sinensis*, native to Southeast Asia, are used: they are prepared by drying the leaves through steam (Japanese method) or by heating (Chinese method) [1,2].

This plant is recognized for its medicinal uses as a stimulant of alertness, a diuretic, an astringent, a digestion promoter, and an enhancer of mental processes. It also contains polyphenols in proportions of 20 % to 36 %, active ingredients with proven antioxidant capacity, and potential cardiovascular, metabolic and neurological benefits [3-5].

On the other hand, there is evidence regarding the toxicity of green tea, which is related to the intake amount. The main adverse effects include hepatotoxicity, gastritis, diarrhea, vomiting, headache, insomnia, tachycardia, and anemia in pregnant women [6,7].

The determination of secondary metabolites in a medicinal plant or in commercial products based on such plant can be carried out through phytochemical screening, which allows for confirming the qualitative presence of alkaloids, flavonoids, phenols and tannins, among others [8]. On

1 Universidad de San Martín de Porres, School of Human Medicine, Instituto de Investigación (Research Institute), Centro de Investigación de Medicina Tradicional y Farmacología (CIMTFAR - Research Center for Traditional Medicine and Pharmacology). Lima, Peru.
2 Universidad de San Martín de Porres, School of Human Medicine, Instituto de Investigación (Research Institute), Programa de Investigación Multidisciplinaria (Multidisciplinary Research Program). Lima, Peru.
3 Universidad de San Martín de Porres, Sociedad Científica de Estudiantes de Medicina (SOCIEM – Scientific Society of Medical Students). Lima, Peru.
4 Colegio Médico del Perú (Peruvian Medical Association), Comité Permanente de Medicina Tradicional, Alternativa y Complementaria (Standing Committee on Traditional, Alternative and Complementary Medicine). Lima, Peru.
5 Sociedad Peruana de Farmacología y Terapéutica Experimental (SOPFARTEX – Peruvian Society of Pharmacology and Experimental Therapeutics). Lima, Peru.
[a] PhD in Medicine, doctor of Medicine, research professor; [b] Bachelor of Medicine, doctor of Medicine; [c] Medical student at Universidad de San Martín de Porres.
*Corresponding author.

the other hand, recognizing the chemical structure and quantity of these compounds requires techniques based on spectrophotometry, high-performance chromatography and magnetic resonance [8]. It is important to recognize the chemical profile of the plant, as it allows inferring and predicting its biological properties [9].

The sale of commercial products based on medicinal plants is subject to the guidelines of the manufacturing process for formally sold products, according to Chapter V of the General Health Law (1997), Articles 88, 89 and 91. It states that a food and/or beverage suitable for consumption is one that complies with the characteristics established by health and quality standards approved by the national health authority, and that the product should only be sold after obtaining sanitary registration and subject to hygienic and sanitary surveillance to protect public health [10].

Medicinal products that are formally sold with sanitary registration in Peru must reach users while complying with quality, safety, and efficacy standards, particularly those marketed for health benefits. This research presents the phytochemical and manufacturing profile of three green tea samples that are formally sold in Lima, Peru.

THE STUDY

This is a descriptive and comparative study conducted at the Centro de Investigación de Medicina Tradicional y Farmacología (CIMTFAR - Research Center for Traditional Medicine and Pharmacology) of the School of Human Medicine at Universidad de San Martín de Porres. Three green tea samples were studied, collected through non-probabilistic and convenience sampling. The collection period was during April 2017.

It was categorized as formal retail sale, according to the following criteria:

- Sale at a shopping center with an operating license: supermarket, store, drugstore or others
- Has sanitary registration
- Is packaged.

To collect the green tea sample, three brands were obtained from a supermarket in the district of Jesús María, province of Lima, and categorized as brands A, B and C.

Regarding the manufacturing characteristics of the products, the presence versus absence of data on the printed packaging was considered, in accordance with the provisions of Article 91 of Chapter V of the General Health Law [10], Article 114 of Title VIII of the Regulation on Health Surveillance and Control of Food and Beverages (approved by Supreme Decree No. 007-98-SA [11], and Article 116 of Title VIII of the Regulation for the Registration, Control and Health Surveillance of Pharmaceutical and Related Products (approved by Supreme Decree No. 010-97-SA) [12]. Based on the foregoing, the study included the following items:

- Commercial name of the product
- Common name of the product
- Scientific name of the product
- Date of packaging
- Expiration date
- Lot number
- Sanitary registration number
- Nutritional information
- Recommended use
- Precautions
- Others (proper sealing, medicinal use information, secondary metabolite information)

The phytochemical screening was performed using the method described by Trease and Evans in 1989 [13-15]. The first step was to obtain the secondary metabolites by exposing the sample to a hydroalcoholic solvent and macerating for one week. The extract was then subjected to the following tests:

- Shinoda test for flavonoids. One milliliter of the test solution is taken, a small magnesium ribbon and 10 drops of concentrated HCl are added, and the mixture is shaken well. The appearance of a pink to cherry red coloration, or red to orange precipitate indicates a positive reaction.
- Dragendorff test for alkaloids. The test solution is placed on filter paper, and then the Dragendorff's reagent is sprayed. The positive reaction is indicated by the formation of a brown halo or an orange precipitate.
- 5 % ferric chloride test for phenols. One ml of the test solution is taken and two drops of ferric trichloride solution (1 % $FeCl_3$) are added. The appearance of a green, blue or black color indicates a positive result.
- 10 % gelatin test for tannins. One ml of the test solution is taken and five drops of gelatin reagent are added to the sample without heating, to prevent gelatin denaturation. Then, five drops of 5 % NaCl are added to enhance the visibility of the reaction, after which it is left to stand for a few minutes. The positive reaction results in a whitish or turbid precipitate.

The chemical reaction was expressed in colorimetric changes of the solution, which were described qualitatively as the presence (or absence) of secondary metabolites: "+++" (abundant), "++" (moderate), "+" (slight), and "-" (absence).

Additionally, the results of this study were compared with to those of two phytochemical screening studies of *Camellia*

sinensis from samples that were cultivated, collected and taxonomically identified, from Cuzco (Peru) [16] and Kano (Nigeria) [17].

The results obtained were collected in an ad hoc form and are presented descriptively in a table.

RESULTS

The study of formally sold green tea *(Camellia sinensis)* demonstrated a homogeneous presence of flavonoids, alkaloids and phenols, but a heterogeneous presence of tannins, with moderate amounts in samples B and C, and slight amounts in sample A (Table 1).

On the contrary, in the referenced and taxonomically identified samples from Cuzco and Kano, the presence of phenols and flavonoids was lower in the formally sold samples.

Upon reviewing the manufacturing characteristics, heterogeneity was observed in the data related to secondary metabolites, as well as in indications for medicinal use, which promote such use both directly and indirectly (Table 1).

Table 1. Comparison of the phytochemical screening and manufacturing characteristics of formally sold green tea (*Camellia sinensis*) samples

	Samples of this study			Reference samples	
	"A"	"B"	"C"	Cuzco, Peru	Kano, Nigeria
Phytochemistry					
Flavonoids	++	++	++	+++	+++
Alkaloids	+	+	+	+	+
Phenols	+	+	+	+++	++
Tannins	+	++	++	++	-
Manufacture					
Common name of the product	Yes	Yes	Yes	NA	NA
Scientific name of the product	Yes	Yes	No	NA	NA
Proper sealing	Yes	Yes	Yes	NA	NA
Packaging date	Yes	Yes	Yes	NA	NA
Sanitary registration	Yes	Yes	Yes	NA	NA
Expiration date	Yes	Yes	Yes	NA	NA
Lot number	Yes	Yes	Yes	NA	NA
Nutritional information	Yes	Yes	Yes	NA	NA
Medicinal use	Yes †	No	Yes ‡	NA	NA
Secondary metabolites	No	Yes*	Yes*	NA	NA

Phytochemistry, legend: (+++) abundant presence; (++) moderate presence; (+) slight presence; (-) absence.
Manufacture, legend: (†) for prevention and treatment, due to hypolipidemic, antihypertensive, hypoglycemic and weight loss effects; (‡) for prevention and treatment, due to weight loss, hypolipidemic and antihypertensive effects; (*) presence of secondary metabolites such as flavonoids, alkaloids, tannins and phenols. NA = not applicable/not evaluated.

DISCUSSION

Traditional medicine attributes several medicinal uses to green tea. The main ones include improving alertness, digestive symptoms and headaches, and promoting weight loss. On the other hand, laboratory studies on the properties of *Camellia sinensis* confirm its stimulation of the central nervous system such as the ergogenic effect and gastric secretagogue effect. It also exhibits hypolipidemic, antiaggregant, angioprotective, anti-inflammatory and antioxidant actions, as well as antibacterial effects. Additionally, a meta-analysis study reported that, in

humans, it may benefit by reducing cardiovascular risk factors such as glycemia and lipids [1,2,4,5,7].

In contrast, laboratory studies of acute and subacute exposures, in the dose range of 2,000 to 2,500 mg/kg of green tea extract, do not demonstrate toxic or lethal effects, suggesting that they would be considered suitable for use in food. However, there are studies in humans that refer a probable association between *Camellia sinensis* intake and hepatotoxicity events, which would be related to excessive oxidative stress in the target organ [1,6,16,18].

The biological properties or effects described for green tea are supported by the presence of its secondary metabolites, such as alkaloids, flavonoids, phenols and others [1-3]. Peruvian regulations do not require a phytochemical screening for granting food sanitary registration for the samples in this study [11,12,19], as confirmed by the labels of two formally sold samples, which report the active ingredients. Furthermore, the phytochemical screening conducted in this study showed the presence of phytochemical compounds with heterogeneous distribution, and in smaller proportion, compared to the reference samples from Cuzco and Kano. Similarly, this has been reported for formally sold products made of Peruvian plants such as *muña* and *maca* [20,21]. Thus, this reality is contrary to the precept of quality, standardization, regulation and surveillance, and could also be reflected in the variability of the potential beneficial or adverse effects of this medicinal beverage.

On the other hand, as corroborated in this study, tea is formally sold as a domestic beverage (either as food product or dietary supplement), with labeling and packaging that include indications for medicinal use contradicting the General Health Law [10], which states the following: "Medicinal plants that are offered without references to therapeutic, diagnostic or preventive properties can be freely marketed." Furthermore, the same law stipulates as follows: "The sale of a medicinal plant as a medicinal product requires stricter guidelines, and the sale of these products must be carried out by personnel who comply with the established health requirements and conditions. Such requirements include phytochemical screening, taxonomic identification, toxicity studies and clinical efficacy studies." The latter is endorsed by the Law on Pharmaceutical Products, Medical Devices and Health Products [22], which states the following: a) "The marketing of herbal medicines, their preparations obtained in the form of extracts, lyophilized forms, distillates, tinctures, decoctions or any other galenic preparation with therapeutic purposes, in the condition of compounded medications, pharmacopeial preparations or drugs, is subject to the requirements and conditions established by the relevant regulations;" and b) "Traditional medicinal plants and other natural resources for medicinal use that are offered without references to therapeutic, diagnostic, or preventive properties can be marketed without sanitary registration."

The World Health Organization (WHO) recognizes and promotes the rational use of products derived from traditional medicine, and proposes clear and direct strategies, including the rule-making, regulation and oversight of traditional medicine, in order to maximize their benefits for people's health [23,24]. Although this study has limitations—such as the sample size, the omission of the quantitative identification of active ingredients, and lack of taxonomic identification—it demonstrates contradictions to Peruvian regulations and WHO recommendations. These facts should alert the authorities, in a more prolific work, in favor and protection of human and consumer health.

In conclusion, a heterogeneous phytochemical profile of tannins was observed in the study samples, while the profile was homogeneous for alkaloids, flavonoids, and phenols. Likewise, manufacturing noncompliance was reported in the study samples according to national regulations.

Acknowledgements: We express our gratitude to the leadership of the School of Human Medicine at Universidad de San Martín de Porres for granting the necessary permits, as well as the faculty and technicians of the CIMTFAR of the School of Medicine at Universidad de San Martín de Porres for the academic, scientific and technical support.

Author contributions: EMM, ASG, and SRM conceptualized, planned and designed the study; conducted a literature search; planned the study workflow; oversaw data management; analyzed the statistical data; prepared tables and figures; interpreted the data; wrote the first draft of the manuscript and critically reviewed the manuscript. Likewise, KYA and BMH participated in the study design, conducted a literature search, contributed to writing the second version of the manuscript, and critically reviewed the manuscript. On the other hand, KCY and EMM participated in the data collection and critical review of the manuscript.

Funding sources: This article was funded by the authors and the CIMTFAR of the School of Human Medicine at Universidad de San Martín de Porres.

Conflicts of interest: The authors declare no conflicts of interest.

BIBLIOGRAPHIC REFERENCES

1. Zhao T, Li C, Wang S, Song X. Green tea (Camellia sinensis): A review of its phytochemistry, pharmacology, and toxicology. Molecules [Internet]. 2022;27(12):3909.
2. Tang G-Y, Meng X, Gan R-Y, Zhao C-N, Liu Q, Feng Y-B, et al. Health functions and related molecular mechanisms of tea components: An update review. Int J Mol Sci [Internet]. 2019;20(24):6196.

3. Olson KR, Briggs A, Devireddy M, Iovino NA, Skora NC, Whelan J, et al. Green tea polyphenolic antioxidants oxidize hydrogen sulfide to thiosulfate and polysulfides: A possible new mechanism underpinning their biological action. Redox Biol [Internet]. 2020;37(101731).
4. Zamani M, Kelishadi MR, Ashtary-Larky D, Amirani N, Goudarzi K, Torki IA, et al. The effects of green tea supplementation on cardiovascular risk factors: A systematic review and meta-analysis. Front Nutr [Internet]. 2023;9:1084455.
5. Unno K, Nakamura Y. Green tea suppresses brain aging. Molecules [Internet]. 2021;26(16):4897.
6. Oketch-Rabah HA, Roe AL, Rider CV, Bonkovsky HL, Giancaspro GI, Navarro V, et al. United States Pharmacopeia (USP) comprehensive review of the hepatotoxicity of green tea extracts. Toxicol Rep [Internet]. 2020;7:386–402.
7. Abiri B, Amini S, Hejazi M, Hosseinpanah F, Zarghi A, Abbaspour F, et al. Tea's antiobesity properties, cardiometabolic health promoting potentials, bioactive compounds, and adverse effects: A review focusing on white and green teas. Food Sci Nutr [Internet]. 2023;11(10):5818–36.
8. Nortjie E, Basitere M, Moyo D, Nyamukamba P. Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: A review. Plants [Internet]. 2022;11(15):2011.
9. Kumar A, Nirmal, Kumar M, Jose A, Tomer V, Oz E, et al. Major phytochemicals: Recent advances in health benefits and extraction method. Molecules [Internet]. 2023;28(2):887.
10. Congreso de la República del Perú. Ley No. 26842 sobre la Ley General de la Salud [Internet]. Diario Oficial El Peruano; 1977. Available from: https://www.insm.gob.pe/departamentos/emergencia/archivos/ ley_general_salud.pdf
11. DIGESA. Decreto Supremo Nº 007-98-SA. Reglamento sobre vigilancia y control sanitario de alimentos y bebidas [Internet]. DIGESA; 1998. Available from: http://www.digesa.minsa.gob.pe/NormasLegales/ Normas/DS007_98.pdf
12. DIGESA. Decreto Supremo N°010-97-SA. Reglamento para el registro, control y vigilancia sanitaria de productos farmacéuticos y afines [Internet]. Diario el Peruano, 1997. Available from: http://www.digesa.minsa.gob.pe/NormasLegales/Normas/DS007_98.pdf
13. Kumar A, Nirmal, Kumar M, Jose A, Tomer V, Oz E, et al. Major phytochemicals: Recent advances in health benefits and extraction method. Molecules [Internet]. 2023;28(2):887.
14. Nortjie E, Basitere M, Moyo D, Nyamukamba P. Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: A review. Plants [Internet]. 2022;11(15):2011.
15. Lock de Ugaz O. Investigación fitoquímica: Métodos en el estudio de productos naturales [Internet]. Pontificia Universidad Católica del Perú; 1994. Available from: https://books.google.com.pe/s?id=N36g2QOccXkC&printsec=frontcover&#v=onepage&q&f=false
16. Palacios Hoyos MF, Pamucena Vargas JE. Efecto antibacteriano del extracto hidroalcohólico de hojas secas de Camelia sinensis L. (te verde) en cultivos de Cutibacterium acnes (Acné Vulgaris), in vitro [Undergraduate thesis]. Lima: Universidad Inca Garcilazo De La Vega; 2019. Retrieved from: http://repositorio.uigv.edu.pe/handle/20.500.11818/5053
17. Lawal TA, Ononamadu CJ, Okonkwo EK, Adedoyin HJ, Shettima ML, Muhammad IU, et al. In vitro and in vivo hypoglycaemic effect of camellia sinensis on alpha glucosidase activity and glycaemic index of white bread. Applied Food Research [Internet]. 2022;2(1):100037.
18. Chengelis CP, Kirkpatrick JB, Regan KS, Radovsky AE, Beck MJ, Morita O, et al. 28-Day oral (gavage) toxicity studies of green tea catechins prepared for beverages in rats. Food Chem Toxicol [Internet]. 2008;46(3):978–89.
19. DIGESA. Texto Único de Procedimientos Administrativos - (TUPA). Procedimiento Registro Sanitario de Alimentos de Consumo Humano [Internet]. Available from: http://www.digesa.minsa.gob.pe/ expedientes/detalles.aspx?id=28
20. Pawer Pucurimay D, Park JS, Roca Moscoso M, Salazar Granara A. Diferencias en la presencia de alcaloides y fenoles de cinco muestras de muña de expendio informal procedentes de mercados populares en Lima - Perú. Horiz Med [Internet]. 2018;18(3):25-9.
21. Palma-Gutiérrez E, Prado-Bravo C, Loja-Herrera B, Salazar-Granara A. Características fitoquímicas de muestras comerciales de maca en tres regiones de Perú. CIMEL [Internet]. 2012;17(2)89-93.
22. Congreso de la República del Perú. Ley No. 29459. Ley De Los Productos Farmacéuticos, Dispositivos Médicos y Productos sanitarios. Diario Oficial El Peruano; 2009. Available from: https:// www.gob.pe/institucion/congreso-de-la-republica/normaslegales/2813441-29459
23. Tácuna-Calderón A, Moncada-Mapelli E, Lens-Sardón L, HuacchoRojas J, Gamarra-Castillo F, Salazar-Granara A. Estrategias de la Organización Mundial de la Salud en Medicina Tradicional y Reconocimiento de Sistemas de Medicina Tradicional. Rev Cuerpo Med HNAAA [Internet]. 2020;13(1):101–2.
24. Organización Mundial de la Salud. Estrategia de la OMS sobre medicina tradicional 2014-2023 [Internet]. Ginebra: OMS; 2013. Available from: https://iris.who.int/bitstream/handle/10665/95008/9789243506098_spa.pdf.

Corresponding author:
Alberto Alcibíades Salazar Granara
Address: Av. El Corregidor 1531, Las Viñas, La Molina, Lima-Perú.
Telephone: 511 365 2300, extension 151
E-mail: asalazarg@usmp.pe

Reception date: July 5, 2023
Evaluation date: July 18, 2023
Approval date: May 5, 2024

ORCID iDs

Enrique Moncada-Mapelli	https://orcid.org/0000-0002-2297-0695
Kristel Yañez-Agapito	https://orcid.org/0000-0002-1465-8175
Brenda Milla-Huerta	https://orcid.org/0000-0001-7737-1350
Stephany Rojas-Monge	https://orcid.org/0000-0002-3990-2162
Kiomi Cerda-Yupanqui	https://orcid.org/0000-0003-1572-6085
Alberto Salazar-Granara	https://orcid.org/0000-0003-1996-3176