



Phenolic Compounds, Free Radical Scavenging Activity And α -Glucosidase Inhibition Properties Of Green, Oolong and Black Sacha Inchi Tea Extract

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Abstract

Sacha Inchi leaves may possess antioxidant function which is naturally rich in bioactive ingredients and has been widely processed into herbal teas that has many benefits to human health. According to the global data from the World Health Organization (WHO), there has been a rise in the frequency of diabetes mellitus (DM) among adults. Sacha Inchi tea was prepared using several methods, with green tea not fermented, oolong partially fermented, and black tea completely fermented. The phenolic compounds (total phenolic content (TPC), total flavonoid content (TFC), tannin), free radical scavenging activity (DPPH) and inhibition properties of α -Glucosidase on green, oolong and black Sacha Inchi tea extract were determined. The green Sacha Inchi tea extract displayed the highest TPC and TFC values among the oolong and black Sacha Inchi tea extracts at a concentration of 500 μ g/mL, with values of 2.10 \pm 0.01 μ g GAE/mL and 3.23 \pm 0.20 μ g QE/mL, respectively. Meanwhile, at a concentration of 500 μ g/mL, Sacha Inchi black tea extract demonstrated strong antioxidant activity by DPPH with radical scavenging activity (%) (89.4 \pm 0.45). Furthermore, at a concentration of 15.625 μ g/mL, black, oolong, and green tea extracts exhibited stronger inhibitory effects on α -Glucosidase (95%, 95%, 92%, respectively) than acarbose (90%). Therefore, the processing steps involved in tea production impact the phenolic compounds, free radical scavenging activity, and the ability to inhibit α -Glucosidase in green, oolong, and black tea extracts.



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Introduction

Sacha Inchi (*Plukenetia volubilis* L.) is a well-known plant by multiple names, including inca nut, mountain peanut, sacha peanut, or Inca peanut. In Malaysia, the cultivation of Sacha Inchi has been introduced by the Rubber Industry Smallholders Development Authority (RISDA) with the aim of boosting farmers' income, as this plant offers numerous potential benefits (Zakaria *et al.*, 2022). This plant originates from the Amazon region in South America. Sacha Inchi is identified by its star-shaped fruit capsule, which changes in color from green to blackish brown as it ripens. Within these capsules, there are edible oval seeds that are dark brown with the size of 1.5–2 cm. These seeds are abundant in lipids, making up 35-60% of their composition, and they are high in omega-3, omega-6, and omega-9 fatty acids. Furthermore, these seeds encompass proteins, comprising approximately 25-30% of their content, which include vital amino acids like cysteine, tyrosine, threonine, and tryptophan. Moreover, these seeds offer vitamin E, flavonoids, minerals, polyphenols, and the shell contains higher amounts of alpha-tocopherol and omega-6 and omega-3 fatty acid levels that are comparable. Additionally, the Sacha Inchi leaves might serve as a source of saponins, terpenoids and phenolic compounds, specifically flavonoids (Wang *et al.*, 2018).

According to Wuttisin *et al.*, (2020), the Sacha Inchi leaves are extensively grown in Thailand, and the resulting products, particularly tea and have already made their way into the market which demonstrating significant antioxidant properties. Additionally, Sacha Inchi leaves are potentially endowed with natural antioxidant capabilities due to their abundance of bioactive components, such as polyphenols (Kittibunchakul *et al.*, 2022). The quantity of polyphenols present in the tea plant can fluctuate, contingent on the specific cultivar, climatic conditions and the methods used during processing (Karaosmanoglu & Kilmartin, 2015). Nevertheless, there is insufficient data available regarding the impact of tea processing stages on chemical compositions and bioactive properties (Ding *et al.*, 2022).

Green tea (unfermented), oolong tea (partially fermented) and black tea (fermented) are the three basic forms of tea based on the degree of fermentation (Wang *et al.*, 2008). The increasing

popularity of tea can be linked to its delightful taste, enchanting aroma, revitalizing effects and the multitude of health benefits it offers (Hu *et al.*, 2014). Green tea is rich in bioactive substances, with polyphenols being the primary component, renowned for their antioxidant characteristics (Trisha *et al.*, 2022). According to Yuan *et al.*, (2011) green tea displays notably elevated levels of polyphenols, whereas black tea contains a comparatively lower number of polyphenols.

In general, tea is subjected to a processing method aimed at deactivating polyphenol oxidase in freshly harvested leaves, thereby preventing enzymatic oxidation (Haw *et al.* 2020). The steam blanching procedure may contribute to preserving the tea's colour, polyphenolic content, and antioxidant characteristics (Ahmed & Stepp, 2012). Apart from steam blanching, the drying temperature plays a crucial role in shaping the quality of tea. The use of intense heat during the drying process has the potential to trigger the activation of low molecular weight natural antioxidants and at the same time, deactivate enzymes that might have caused deterioration, ultimately improving the tea's overall quality (Jeong *et al.* 2004).

The leaves of Sacha Inchi have been widely processed into herbal teas which have many benefits to human health (Silalahi., 2022). Despite the various benefits associated with its consumption, the utilization of Sacha Inchi leaves remains restricted or limited. There is potential for exploring the use of Sacha Inchi leaves in tea production to enhance their utilization and added value. Therefore, the objective of the present investigation was to examine the phenolic compounds, examine the free radical scavenging capabilities, and determine the α -Glucosidase inhibition characteristics of extracts derived from green, oolong, and black Sacha Inchi.

Materials and Methods

Fresh Sacha Inchi leaves were harvested from the Sacha Inchi plantation. The selection process involved picking vibrant, undamaged and fully matured leaves. These freshly plucked Sacha Inchi leaves were then carefully stored in airtight plastic containers to prevent any bruising or wilting. Following this, the leaves were thoroughly washed with clean water and underwent further processing tailored to the desired final product. Additionally,

commercial green tea, oolong tea, black tea (all containing *Camellia sinensis*), and dried stevia were procured from the local market.

Production of Green Tea, Oolong Tea and Black Tea from Sacha Inchi Leaves

Tea is typically categorized as green, black, or oolong, depending on the processing method it undergoes and the extent of enzymatic oxidation. Green tea is left unfermented, oolong tea is partially fermented and black tea is fully fermented. This study involved the creation of three tea varieties: green tea, oolong tea, and black tea, all made from Sacha Inchi leaves using distinct processing methods and varying degrees of fermentation. It's essential to recognize that tea consists of a blend of leaves from the *Camellia sinensis* plant (Awadalla *et al.* 2011). Thus, in this study, the tea composition was formulated using the following ratios; 50% of commercial Green, Oolong and Black tea (which consists of *Camellia sinensis* plant), 45% Sacha Inchi tea leaves (Green, Oolong and Black) and 5 % of the commercial dried stevia.

Processing of Green Tea from Sacha Inchi leaves

The production of green tea in this study was modified from the method outlined in KC *et al.* (2020) with some modifications. The process included steaming Sacha Inchi leaves at 100°C for 14 minutes, followed by a 15-minute cooling period and a subsequent 15-minute hand-rolling phase. After this, the leaves were dried using an electric oven (Luxury Electric Deck Oven, China) at 110°C for 20 minutes.

A final 30-minute rolling session was carried out after drying to complete the green tea production. The weight of the final product was recorded, and it was vacuum-sealed using an Automatic Heavy Duty Double Chambers Vacuum Packaging Machine (Taiwan). For the analytical process, a mixture was prepared by combining 50% commercial green tea, 45% Sacha Inchi green tea leaves, and 5% commercial dried stevia.

Processing of Oolong Tea from Sacha Inchi Leaves

Oolong tea of Sacha Inchi was produced according to Wong *et al.* (2022) with some modifications. The process began with an indoor withering of Sacha Inchi leaves. During this step, the leaves were

evenly laid out on a tray and left at room temperature for 2 hours, with two turning treatments applied. Subsequently, the leaves underwent parching and were then subjected to fermentation in a laboratory dryer (Floor Model Laboratory Dryers FDD-720, Malaysia) at 25°C for 2 hours. Following fermentation, the leaves were dried in an electric oven (Luxury Electric Deck Oven, China) at 160°C for 20 minutes.

Next, they were manually rolled for a duration of 5 minutes and then subjected to additional drying in the same electric oven at 80°C for another 20 minutes. Finally, the leaves were hand-rolled once more, completing the production of oolong tea. The final weight was recorded and the oolong tea was vacuum-sealed to maintain its moisture content, utilizing an Automatic Heavy Duty Double Chambers Vacuum Packaging Machine (Jaw Feng Machinery, Taiwan). For analytical purposes, a blend was created, consisting of 50% commercial oolong tea, 45% Sacha Inchi oolong tea leaves, and 5% commercial dried stevia.

Processing of Black Tea from Sacha Inchi Leaves

Black tea of Sacha Inchi leaves was produced according to KC *et al.*, (2020) with some modifications. To begin, Sacha Inchi leaves were thinly spread on trays for indoor withering at room temperature for a period of 2 hours. Following this, the leaves were hand-rolled on a tabletop surface for a duration of 25-35 minutes. The fermentation process occurred in a laboratory dryer (Floor Model Laboratory Dryers FDD-720, Malaysia) for 14 hours at 25°C.

After the completion of fermentation, additional rolling took place, followed by drying in an electric oven (Luxury Electric Deck Oven, China) at 200°C for 4 minutes. Once all these steps were concluded, the black tea derived from Sacha Inchi leaves was produced and vacuum-sealed using Vacuum Packaging Machine (Jaw Feng Machinery, Taiwan). For the purpose of analysis, a blend was prepared, combining 50% of commercial black tea, 45% Sacha Inchi black tea leaves, and 5% of commercially dried stevia.

Preparation of Extracts from Sacha Inchi Tea

Maceration procedure was performed by weighing 30g of tea sample consisting of ratios; (50 commercial tea: 45 Sacha Inchi leaves: 5 commercial dried

stevia) respectively and 300 ml of ethyl acetate (Milan, 2011). The mixture was placed in a 500 mL beaker and left undisturbed in the dark at room temperature for 24 hours. Subsequently, the supernatants were collected following filtration via a Grade 1 Whatman® qualitative filter paper once the extracts had been filtered. Pooled supernatants were condensed in a rotary evaporator (Heidolph Rotary Evaporator, German) at 60°C under reduced pressure. Before being used, the extract was collected and kept in a chiller at 5°C.

Phenolic Compounds analysis of Green, Oolong and Black Sacha Inchi Tea Extract

Preparation of Standard Solution

The preparation of gallic acid and quercetin standard solutions followed the method outlined by Shirazi *et al.*, (2014). Gallic acid (1 g) was dissolved in 100 ml of methanol, yielding a 1% concentration of Gallic acid. This solution is referred to as the standard 1 solution. Similarly, 1 g of Quercetin was dissolved in 100 ml of methanol to obtain a 1% concentration of Quercetin in a standard 2 solution.

Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu colorimetric method was used to determine the TPC in Sacha Inchi extracts as outlined by Fuad *et al.*, (2020). Initially, a stock solution of the extracts was produced at a concentration of 1 mg/mL in ethyl acetate. Subsequently, this solution was diluted to create three distinct concentrations (125, 250, and 500 µg/mL). A microplate reader (Thermo Scientific™ Multiskan™ FC Microplate Photometer, Finland) was used and the absorbance was measured at a wavelength of 765 nm. The total phenolic content was calculated using the gallic acid standard curve, and the findings were represented as milligrams of gallic acid equivalent per microgram of extract. The calibration equation for gallic acid was $y = 0.8409x + 0.0094$ ($R^2 = 0.9991$). Every experiment was carried out in triplicate.

Determination of Total Flavonoid Content (TFC)

The TFC of the sample was measured using the techniques outlined in Milan (2011) and Maheshkumar (2012) studies. Extract stock solution was produced at 1 mg/mL in ethyl acetate and was then diluted to various concentrations (125, 250, and 500 µg/mL). A microplate reader (Thermo Scientific™ Multiskan™ FC Microplate Photometer, Finland) was used to measure the absorbance at 415

nm. The result was expressed as mg of quercetin equivalents per microgram of extract. The calibration equation for gallic acid was $y = 0.4316x + 0.0449$ ($R^2 = 0.9973$). All the experiments were performed in triplicate.

Analysis of Tannins Concentration

Preparation and Standardization of the Reagent

Two primary reagents employed for tannin quantification were indigo carmine and potassium permanganate (KMnO₄). The indigo carmine powder (6 g) was dissolved in 500 mL of distilled water to produce the indigo carmine solution and subsequently boiling it. After the mixture had cooled, 50 ml of a 95% H₂SO₄ solution was added. The resultant mixture was then diluted with distilled water until reaching a total volume of 1 litre, followed by filtration using qualitative filter paper. Potassium permanganate powder (3.3 g) were dissolved in 1 L of distilled water to get a KMnO₄ solution. Titration was used to standardize the solution with oxalic acid. The standardization resulted in 1 ml of 0.1 N KMnO₄ equalling 0.0067g oxalic acid (Wangiyana *et al.*, 2021).

Qualitative Estimation of Tannin

A preliminary examination of Sacha Inchi tea samples containing tannins was conducted. To identify the presence of tannins, three drops of an aqueous solution with a 5% (w/v) ferric chloride content were added to 1 mL of green tea, oolong tea, and black tea made from Sacha Inchi leaves. The greenish precipitate formation was observed which indicates the presence of tannins in the samples. Following a positive test result, a quantitative evaluation of tannin content was performed (Wangiyana *et al.*, 2021).

Quantitative Estimation of Tannin

Tea samples were titrated for quantitative estimation with a standard KMnO₄ solution, based on Wangiyana *et al.*, (2021) with some modifications. A stock solution of the extracts was initially formulated in ethyl acetate at a concentration of 1 mg/mL. Subsequently, this solution was diluted into three distinct concentrations (125, 250 and 500 µg/mL). In a 250 mL conical flask, 2.5 mL of tea samples and 2.5 mL of indigo carmine solution were mixed. The resulting mixture was then diluted with 75 mL of distilled water. Next, titration was performed with standardized KMnO₄ until the solution's blue colour turned to green. A few additional drops were added

until the solution turned golden yellow. The indigo carmine solution (2.5 mL) was mixed with 75 mL of distilled water for the blank test, followed by titration. To ensure accuracy, triplicate samples were used for evaluation.

The tannin content (T%) in the sample was determined using the following formula:

$$T (\%) = (V - V_0) \times 0.004157 \times 2.5 / (g \times 80) \times 100\%$$

V represents the volume of 0.1 N KMnO_4 utilized for titrating the sample (mL), V_0 denotes the volume of 0.1 N KMnO_4 employed for titrating the blank sample (mL), 0.004157 signifies the tannins equivalent in 1 mL of 0.1 N KMnO_4 , 2.5 corresponds to the volume of the extraction solvent used for the sacha inchi tea extract sample (mL), g represents the mass of the sample selected for analysis (g), and 80 indicates the total sample volume (mL).

Free Radical Scavenging Activity Analysis of Green Tea, Oolong Tea and Black Tea Extract 2,2-Diphenyl-1-Picrylhydrazyl (Dpph) Assay

The assessment of free radical scavenging activity in the sample extract was conducted using a method adapted by Mensor *et al.*, (2001) and Dudonne *et al.*, (2009). Initially, a 1 mg/mL stock solution of the extracts in ethyl acetate was produced. Following that, the solution was diluted into six different concentrations (15.625, 31.25, 62.5, 125, 250, 500, and 1000 $\mu\text{g/mL}$). For the control, a blank solution comprising ethanol and DPPH was prepared. In a 96-well plate, 150 μL of a 0.1 mM DPPH working solution was added to the sample (50 μL), followed by thorough mixing. The plate was then placed in a dark environment and left undisturbed for 30 minutes. Afterward, the change in absorbance was measured at 515 nm using a microplate reader (Thermo Scientific™ Multiskan™ FC Microplate Photometer, Finland).

Ascorbic acid served as the standard in this analysis. Three sets of measurements were conducted and the percentage of DPPH scavenging activity was determined using the following formula, where A_c represents the absorbance of the control and A_s stands for the absorbance of the sample.

$$\text{DPPH radical scavenging activity (\%)} = (A_c - A_s) / A_c \times 100\%$$

α -Glucosidase

Preparation of Phosphate Buffer (0.1 M Phosphate Buffer pH 7.0)

Following the procedure outlined by Xing *et al.* (2017) with some modification, the preparation of a 0.1 M phosphate buffer at pH 7.0 entailed the following steps: Initially, 35.5 grams of sodium phosphate dibasic were dissolved in 500 mL of distilled water, resulting in the creation of a 0.5 M sodium phosphate dibasic stock solution. Subsequently, by diluting 80 mL of the 0.5 M sodium phosphate dibasic solution with distilled water to a final volume of 400 mL, a 0.1 M sodium phosphate dibasic solution was produced. Approximately 30 grams of anhydrous sodium phosphate monobasic were dissolved in 500 mL of distilled water to produce the 0.5 M sodium phosphate monobasic stock solution. Then, the 0.5 M sodium phosphate monobasic stock solution was then diluted with distilled water to a final volume of 150 mL resulting in a 0.1 M sodium phosphate monobasic solution. The 0.1 M phosphate buffer solution was prepared by adding the required amount of 0.1 M sodium phosphate monobasic solution to the 0.1 M sodium phosphate dibasic solution until a pH of 7.0 was reached.

Preparation of p-nitrophenyl- α -D Glucopyranoside (PNPG) Solution

The preparation of PNPG solution was based on a method by Mohiuddin *et al.*, (2016) with some modification. The PNPG powder (0.0023g) was dissolved in 15 mL of phosphate buffer (0.1 M, pH 7.0).

Preparation of α -Glucosidase Solution (0.2 unit/mL)

Preparation of working solution for 25 μL was used in each well which calculated according to the following formula:

$$M_1 \times V_1 = M_2 \times V_2$$

Where M_1 is the concentration of alpha glucosidase enzyme which is 100 unit/mL, V_1 Volume needs to be taken from 100 unit/mL, M_2 is the concentration for working solution and V_2 is the total volume needed of 96 well plates.

α -Glucosidase Inhibition Activity Assay

According to Wan Nadilah *et al.* (2019), the glucose-lowering ability of the leaf extracts was evaluated by

determining their α -glucosidase inhibitory activity. The assay of α -Glucosidase inhibitory activity in green tea, oolong tea, and black tea was conducted following the procedure outlined by Yilmazer-Musa *et al.* (2012) with some modification. A stock solution of the extracts was initially formulated at a concentration of 1 mg/mL in ethyl acetate.

Subsequently, this solution was then diluted to obtain seven different concentrations (15.625, 31.25, 62.5, 125, 250, 500, and 1000 μ g/mL). The 100 unit/mL of α -glucosidase from *S. cerevisiae* was diluted into 0.2 unit/mL in 3 μ L phosphate buffer (pH 7.0). The sample extract (10 μ L) and acarbose as a control were added into well A and B. Followed 10 μ L distilled water (well B-H) and 10 μ L of serial dilution. The reaction was initiated by introducing 25 μ L of pNPG as the substrate, along with 50 μ L of phosphate buffer, and 25 μ L of α -Glucosidase into all wells of a 96-well plate. These plates were then incubated at 37°C for a duration of 30 minutes.

Following this initial incubation, the plate underwent an additional 15-minute incubation at 37°C, and the reaction was halted by adding 100 μ L of 1 M sodium carbonate (Na_2CO_3). The progress of the reaction was monitored by measuring the change in absorbance at 410 nm using a microplate reader.

The α -Glucosidase inhibitory activity was presented as inhibition percent and was calculated as in the equation below:

$$GI\% = (1 - A_s / A_c) \times 100$$

where GI represents α -Glucosidase inhibition, where A_s and A_c correspond to the absorbance of the sample and control, respectively. Each incubation process was repeated three times, and the presented results represent the average value along with the standard deviation derived from these triplicate determinations.

Statistical Analysis

The total phenolic content, total flavonoid content, free radical scavenging activity (DPPH) and α -Glucosidase inhibition activity assay were performed in triplicate for each sample and concentration. The measurements obtained were averaged to calculate the mean value for each assay. The results were presented with \pm standard deviation (SD) and statistical analysis was conducted using Statistical Package for the Social Science (SPSS) Software version 20.0. A two-way (ANOVA) and appropriate comparison were made using the Duncan test with significance defined as ($p < 0.05$).

Table 1: Total Phenolic Content (μ g GAE/g extract) of Sacha Inchi tea extract

Concentration (μ g /mL)	Sample		
	Green Tea	Oolong Tea	Black Tea
125	1.84 \pm 0.02 ^{aA}	1.75 \pm 0.00 ^{bA}	1.61 \pm 0.01 ^{cA}
250	2.00 \pm 0.01 ^{aB}	1.85 \pm 0.00 ^{bB}	1.75 \pm 0.01 ^{cB}
500	2.10 \pm 0.01 ^{aC}	1.98 \pm 0.01 ^{bC}	1.83 \pm 0.01 ^{cC}

The data is presented as the mean \pm SD based on triplicate determinations (n=3).

Mean values within the same row with distinct lowercase letters are statistically significant ($p < 0.05$).

Mean values within the same column with different uppercase letters are statistically significant ($p < 0.05$).

Results and Discussion

Total Phenolic Content (TPC)

Phenolic compounds primarily encompass flavonoids, phenolic acids, stilbenes, coumarins, and tannins, primarily serving as antioxidants that counteract free radicals (Islam *et al.*, 2015). The

assessment of total phenolic content was conducted through the utilization of the Folin-Ciocalteu method, which relies on colorimetry and reactions of electron transfer between the Folin-Ciocalteu reagent and phenolic compounds. The total phenolic content in the ethyl acetate-extracted tea samples fell within

the range of 1.61 to 2.10 $\mu\text{g GAE/mL}$, in different concentration as indicated in Table 1.

The content of phenolic compounds varied substantially ($p > 0.05$) between concentrations at different type of tea which the subset represents in lowercase alphabet (a, b, c) and between the same group of tea sample with different concentration which represent uppercase subset (A, B, C). From the table, at concentration 500 $\mu\text{g/mL}$, green tea, oolong tea and black tea has the highest value of phenolic content ($2.10 \pm 0.007 \mu\text{g GAE/mL}$, $1.98 \pm 0.013 \mu\text{g GAE/mL}$, and $1.83 \pm 0.005 \mu\text{g GAE/mL}$). This finding was aligned with study reported by Abdullah & Mazlan (2020), indicate that all green tea samples have a higher phenolic content in comparison to herbal teas. The variations in these findings can be traced back to the prevalence of catechins, the principal phenolic compound in green tea samples. Furthermore, elements like the initial leaf processing before drying, the region of cultivation, and the environmental conditions can also impact catechin levels. Moreover, the time of year, climate, and maturity of the tea leaves significantly affect the overall composition of the teas.

According to Yang & Liu (2013), the overall phenolic content in tea is typically high across various types of tea. Nevertheless, specific varieties, such as green tea and black tea, are recognized for their elevated phenolic content compared to other types. Green tea, in particular, boasts a wealth of phenolic compounds, most notably catechins like epigallocatechin, epigallocatechin gallate (EGCG), epicatechin and epicatechin gallate. These catechins serve as potent antioxidants and green tea's antioxidants play a significant role in the numerous health benefits linked with it. Black tea, on the other hand, also contains phenolic compounds, but its oxidation process during production diminishes the overall catechin content compared to green tea. Nonetheless, black tea still retains substantial quantities of other phenolic compounds, such as theaflavins and thearubigins, which contribute to its distinctive flavor and health attributes.

Other types of tea, such as white tea and oolong tea, also contain phenolic compounds but in varying amounts. White tea, being minimally processed, retains a relatively higher phenolic content, whereas

oolong tea is in between green tea and black tea in terms of phenolic levels. It's important to highlight that the exact phenolic content can fluctuate due to factors such as tea quality, cultivation conditions, processing methods, and brewing approaches. Nevertheless, all types of tea are generally acknowledged as valuable sources of phenolic compounds (Yang & Liu, 2013). According to Roslan *et al.*, (2020), rapid and brief heating may result in the deactivation of oxidative enzymes, resulting in greater retention of phenolic chemicals and thus the increased of TPC in the black tea could be linked to the generation of phenolic compounds through non-enzymatic conversions among phenolic molecules, likely influenced by the presence of phenolic precursors.

Total Flavonoids Content (TFC)

Tea is versatile in its consumption, with options ranging from brewing loose leaves or using tea bags to opting for ready-to-drink varieties. Additionally, tea extracts can be tailored to suit various needs, coming in a range of physical forms. These tea extracts come in various forms, including liquid preparations like potent infusions, semi-solid concentrates, and purified catechins. Potent infusions are produced by steeping tea leaves in alcohol/water mixtures, while semi-solid concentrates are produced by reducing the water content of the strong infusion to about 20-25%. For tea extract powders or dry extracts, concentrated infusions are transformed through spray drying, leading to solid concentrations of 40-50% (Wang *et al.*, 2000). According to Dwyer & Peterson (2013), flavonoids belong to a group of phenolic compounds commonly encountered in a variety of plant-based foods, with tea standing out for its notably high levels of these compounds. Among the non-nutrient bioactive components in our diets, flavonoids are well-known for their exceptional diversity and wide distribution.

Table 2 shows the TFC in samples of green, oolong and black tea extract of Sacha Inchi leaves. The range of the total flavonoid content extracted with ethyl acetate was 0.56 to 3.23 $\mu\text{g QE/mL}$. Tea fermentation includes the oxidation of flavonoids within tea leaves, which is triggered by the release of intracellular polyphenol oxidase. This transformation impacts both the color and flavor profile of the final product, as well as the composition of flavonoids. Catechins are sensitive to oxidation during the

manufacture of black tea, resulting in its distinctive color and flavor characteristics that define black tea. Traditionally, the pigments in black tea have been classified into orange-hued theaflavins. Consequently, in black tea, four primary theaflavins are discernible: theaflavin 3'-gallate, theaflavin

3,3'-digallate, theaflavin and theaflavin 3-gallate. These theaflavins emerge from the interaction between quinones originating from a foundational catechin and a gallocatechin (Dwyer & Peterson, 2013).

Table 2: Total flavonoids content ($\mu\text{g QE/g}$ of extract) of Sacha Inchi tea extracts

Concentration ($\mu\text{g/mL}$)	Sample		
	Green Tea	Oolong Tea	Black Tea
125	$0.94 \pm 0.03^{\text{aB}}$	$0.56 \pm 0.06^{\text{bB}}$	$0.82 \pm 0.03^{\text{aB}}$
250	$1.38 \pm 0.07^{\text{aB}}$	$0.88 \pm 0.04^{\text{aB}}$	$1.05 \pm 0.16^{\text{aB}}$
500	$3.23 \pm 0.20^{\text{aA}}$	$2.49 \pm 0.29^{\text{aA}}$	$3.17 \pm 0.07^{\text{aA}}$

The data is presented as the mean \pm SD based on triplicate determinations (n=3). Mean values within the same row with distinct lowercase letters are statistically significant ($p < 0.05$). Mean values within the same column with different uppercase letters are statistically significant ($p < 0.05$).

Furthermore, green tea is unfermented, whereas oolong tea is partially fermented and black tea is fermented. According to Fang *et al.* (2021), distinct tea processing procedures result in different biochemical changes inside the tea leaves. The tea production process involves both enzymatic and nonenzymatic transformations, leading to intricate alterations in tea flavonoids and resulting in the differentiation of tea varieties (Peterson *et al.*, 2005). Throughout all stages of tea production, including withering, 'killing-green,' 'shaking-green,' yellowing, fermentation, microbiological fermentation, and drying, significant modifications occur in tea flavonoids. Tea leaves typically contain catechins as the predominant flavonoids, but their concentrations vary notably among different types of tea. Factors like oxygen levels, system pH, temperature, and moisture content during tea processing contribute to the variability in tea catechin profiles (Ananingsih *et al.*, 2013). As a result, tea catechins can undergo substantial alterations influenced by temperature, time and their interaction (Ye *et al.*, 2018).

In this study, quercetin served as the standard due to its prevalence as the most common flavanol typically found in tea. The results indicated that green tea exhibited the highest flavonoid content ($3.23 \pm 0.197 \mu\text{g QE/mL}$), slightly surpassing that of black tea ($3.17 \pm 0.074 \mu\text{g QE/mL}$). However,

these findings contrasted with those reported by Rahman *et al.* (2021), where a notable disparity in the average flavonoid content between black and green tea was observed. In their study, black tea displayed an average flavonoid content of 61.82 mg QE/g dry extract, whereas green tea had a lower average of 31.85 mg QE/g dry extract. This divergence can be attributed to the fact that green tea undergoes minimal processing and oxidation, which helps preserve its flavonoid content. Notably, green tea predominantly contains key flavonoids such as catechins, including epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG). In contrast, black tea experiences significant oxidation during its production, resulting in changes to the composition and quantity of flavonoids. The primary flavonoids in black tea are theaflavins and thearubigins, and their levels are influenced by the degree of oxidation (Bag *et al.*, 2022).

Tannin Concentration (%)

Qualitative Estimation of Tannin

Based on Table 3, all the sample shows positive results on qualitative estimation where (+) indicates that there was tannin present in the tea extract. Tannins are high molecular weight phenolic polymers commonly present in grapes, tea, and legumes (Saxena *et al.*, 2013). Studies have shown

that phytoconstituents classified as tannins have also demonstrate radical scavenging abilities significant antioxidant activity, and certain tannins (Ghosh,2015).

Table 3: Tannin screening of Sacha Inchi Tea Extract

Concentration ($\mu\text{g}/\text{mL}$)	Sample		
	Green Tea	Oolong Tea	Black Tea
125	+	+	+
250	+	+	+
500	+	+	+

Positive (+) signifies the detection of tannin in the tea extract.

Quantitative Estimation of Tannin

Tannin is an essential component that has been recognized as a quality standard element in traditional tea preparations (KC *et al.*, 2020). Plant tannins are polyphenols that are divided into condensed and hydrolysable tannins with a wide structural diversity and high polymerization levels

(Kardel *et al.*, 2013). The tannin concentrations in several forms of traditional tea, such as green tea, oolong tea, and black tea, vary significantly. These varying tannin levels are responsible for the distinct tastes and flavours found in each type of conventional tea product (Khasnabis *et al.*, 2015).

Table 4: Tannin concentration in tea sample (%)

Concentration ($\mu\text{g}/\text{mL}$)	Sample		
	Green Tea	Oolong Tea	Black Tea
125	20.54 \pm 0.00 ^{cB}	41.07 \pm 10.27 ^{bA}	54.76 \pm 5.93 ^{aA}
250	25.36 \pm 0.00 ^{cA}	32.12 \pm 2.93 ^{bA}	45.64 \pm 5.07 ^{aA}
500	13.16 \pm 1.43 ^{aC}	18.10 \pm 1.43 ^{bB}	27.15 \pm 2.47 ^{cB}

The data is presented as the mean \pm SD based on triplicate determinations (n=3). Mean values within the same row with distinct lowercase letters are statistically significant ($p < 0.05$).

Mean values within the same column with different uppercase letters are statistically significant ($p < 0.05$).

Tannins, a widely distributed class of phenolic antioxidants, can be found in various plant sources like barks, leaves, fruits, and vegetables. These compounds have gained attention for their potential medicinal and therapeutic properties. Among them, tannic acid stands out as an exceptional compound with robust antioxidant capabilities. The structural feature responsible for tannic acid's antioxidant properties consists of its hydrophobic central core, which includes glucose, and its hydrophilic shell. Tannic acid is characterized by ten galloyl groups and is water-soluble. Phenolic compounds are

renowned for their antioxidant activity due to their redox properties, enabling them to counteract oxygen radicals by supplying hydrogen to neutralize reactive oxygen species (Ghosh, 2015).

Based on Table 4, black tea exhibited the highest tannin concentration, ranging from 27.15% to 54.76%, in contrast to oolong and green tea extracts, with green tea containing the lowest tannin content. These findings align with those of Khasnabis *et al.* (2015), who reported the tannin concentration of black tea ranges from 11.76% to 15.14%,

with an average of 13.36%. Tea tannin, a type of polyphenol found in tea leaves, differs from tannic acid. Additionally, Kaur *et al.* (2015) noted that the total polyphenol content in green tea (3.066 ± 1.911 mg TAE/g) was significantly higher than that in black tea (0.72 ± 0.55 mg TAE/g) ($p < 0.05$).

Furthermore, the findings clearly indicate that black tea exhibits the highest tannin content among oolong and green teas. Nevertheless, when concentration is taken into account, a reverse relationship emerges, with greater concentration leading to a reduction in tannin levels within the tea. Hence, the extraction of tannins into the tea infusion can also be influenced by the brewing time and temperature. According to Khasnabis *et al.*, (2015), although black tea tannins offer potential health advantages, it is crucial to exercise restraint in consumption. Excessive tannin intake can lead to adverse effects, including interference with iron absorption and inducing digestive discomfort in individuals with heightened sensitivity.

Hence, the quantity of tannin in tea has a substantial influence on its coloration. As stated by Ghosh, (2015), there is a direct relationship between tea's blackness and the concentration of tannins it contains. In other words, teas with a darker colour have a greater tannin concentration. Tannins are polyphenolic compounds present in a variety of plant-based foods and drinks, including tea. Tannins contribute to black tea's distinctive astringency and bitterness. The higher tannin content in black tea is primarily due to the processing methods involved in its production. During the production of black tea, tea leaves undergo withering, rolling, and oxidation. The oxidation process, in particular, leads to the formation and accumulation of tannins in the tea leaves.

Kumari and Jain (2015) have reported that the consumption of food products containing tannins differs across regions. Their research indicates that in India, the intake of tannins ranges between 1.5 and 2.5 grams per day, varying by the area. On the other hand, the United States has an average tannin consumption of around 1 gram per day. It is worth nothing that a daily tannin intake within the range of 1.5-2.5 grams is considered safe and does not lead to any adverse effects.

Furthermore, according to the study by Bone & Mills (2013), excessive amounts of tannins result in an intensified astringency on mucous membranes, which can cause irritation. However, prolonged consumption of tannins inhibits the activity of digestive enzymes, particularly the membrane-bound enzymes found on the mucous membranes of the small intestine. In conclusion, variations in tannin levels among various tea samples can likely be attributed to differences in the manufacturing process, tea leaf aging, or variations in climate and soil conditions (Khasnabis *et al.*, 2015).

Free radical Scavenging Activity (2,2-diphenyl-1-picrylhydrazyl, DPPH)

The levels of free radical scavenging activity were assessed at various concentrations, ranging from 15.625 to 500 $\mu\text{g/mL}$ for Sacha Inchi tea extract (green tea, oolong tea and black tea) (Figure 1). The DPPH radical is a stable free radical that is widely used to test antioxidant activity (Chen *et al.*, 2012). According to Silalahi (2022), the leaf of Sacha Inchi extract contains phenolic compounds, steroids, and terpenoids that are associated to their ability as antioxidants which are declared equivalent to ascorbic acid ranging from 59.31 to 97.76 EAA/g, while DPPH test value is 62.8% to 88.3%.

Figure 1 shows that the most significant DPPH radical scavenging activity was found in black, oolong, and green tea extract samples at 500 $\mu\text{g/mL}$ with the percentages of scavenging activity at 89.4, 48.9 and 41.4% respectively. Thus, the black tea demonstrated the widest range of percentage scavenging activity (20.4 to 89.4% $\mu\text{g/mL}$) of all three tea samples at five different concentrations and the data was higher than the finding of Silalahi (2022). Moreover, Kumar *et al.* (2014) revealed that Sacha Inchi extracts from Quito, Ecuador exhibited DPPH radical scavenging activities of up to 19% within a 1.0 mL sample. However, in the present study of DPPH show that the percentage of scavenging activity was higher starting from 9.1% up to 89.4% at concentration 15.625 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$ respectively compared to the study done by Kumar *et al.* (2014). The variation is due to the temperature, storage method, and chemicals used for extraction all have a major influence on Sacha Inchi's ability to operate as an antioxidant (Silalahi, 2022).

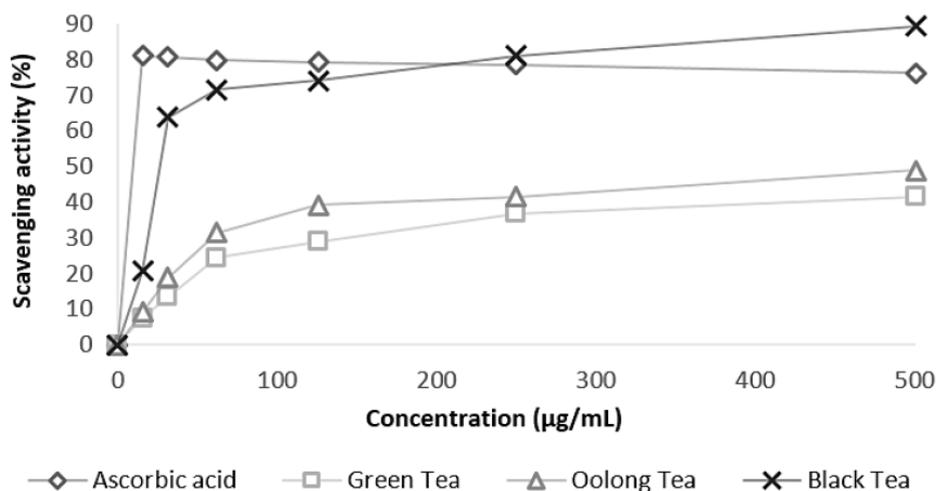


Fig. 1 : DPPH radical scavenging activity (%) of tea extract at different concentration

According to Meng *et al.* (2018), the increase in antioxidant activity observed in samples exposed to higher drying temperatures may be attributed to the release of phytochemical compounds that were previously bound, which occurs as cellular components degrade and new compounds with enhanced antioxidant properties are formed. This phenomenon could explain the heightened antioxidant activity observed in samples dried at elevated temperatures, as seen in the case of Sacha Inchi black tea dried at 200°C. Furthermore, the increase in trend by the black tea sample was correlate with Chen *et al.* (2012), as the percentage scavenging rate of black tea from *Camellia sinensis* L. increased with increasing concentration (1.7 µg/mL to 13.3 µg/mL). However, in this study three types of tea were produced from Sacha Inchi leaves. Thus, different concentrations from tea extract were compared to make comparative evaluation on the effect on antioxidants and black tea extract shown higher percentages of scavenging activity range from 20.4 to 89.4% µg/mL.

α-Glucosidase Inhibition Activity Assay

Glycosidases, enzymes that break glycosidic bonds in polysaccharides and glycoconjugates, play important roles in a variety of biological activities, including carbohydrate digestion, lysosomal glycoconjugate breakdown, and glycoprotein post-translational modification. Within carbohydrate digestion, α-Amylase and α-Glucosidase play pivotal roles. α-Amylase facilitates the conversion of dietary carbohydrates into disaccharides. Subsequently,

α-Glucosidase operates in the small intestine, breaking down disaccharides (e.g., maltose and sucrose) into monosaccharides like glucose before absorption. Inhibiting α-Amylase and α-Glucosidase can moderate the pace of starch digestion, thereby mitigating the post-meal rise in blood glucose levels. Consequently, these enzymes, α-Amylase and α-Glucosidase, represent potential targets for managing postprandial hyperglycaemia in individuals with type 2 diabetes (Xu & Wang, 2019).

In the present study, α-Glucosidase inhibition activity on Sacha Inchi tea extract of green, oolong and black tea at different concentrations was identified. In Figure 2, it is evident that black, oolong, and green tea extracts exhibited a more effective inhibitory impact on α-Glucosidase, with inhibitory rates of 95%, 95%, and 92%, respectively. At a concentration of 15.625 µg/mL, these rates exceeded those of acarbose (90%). However, green tea extract shows that up to concentration 250 µg/mL (83%) it is more potent than acarbose with same concentration compared to oolong and black tea extract which showed their inhibitory effects were only potent than acarbose up to 125 µg/mL of concentration.

Tea is well-known for its multiple health advantages. Several studies have shown that black tea has anticancer and antioxidative characteristics, aids in lowering postprandial blood sugar and blood fat levels, decreases the risk of cardiovascular disease, and helps to prevent obesity (Pan *et al.*, 2013). As reported by Li *et al.* (2013), the primary

constituents found in black tea include polyphenols, flavanol or flavone compounds, theaflavins and thearubigins. Additionally, research conducted by Zhang *et al.* (2010) has indicated that the majority of α -Glucosidase inhibitors derived from tea are tea polyphenols or polyphenol polymers.

Therefore, as indicated by the results presented in Figure 2, it is possible that black tea could serve as an inhibitor of α -Glucosidase, potentially opening avenues for the development of innovative

medications and functional foods aimed at diabetes prevention and treatment. However, it's worth noting that the trend in percentage inhibition of green, oolong, and black tea extracts using ethyl acetate from Sacha Inchi tea leaves, as observed in the results, contradicts the findings of Huang *et al.* (2013). Their study reported an increase in the inhibitory effect of the ethyl acetate fraction from 20.43% to 84.77% with an increase in concentration from 4 μ g/mL to 125 μ g/mL.

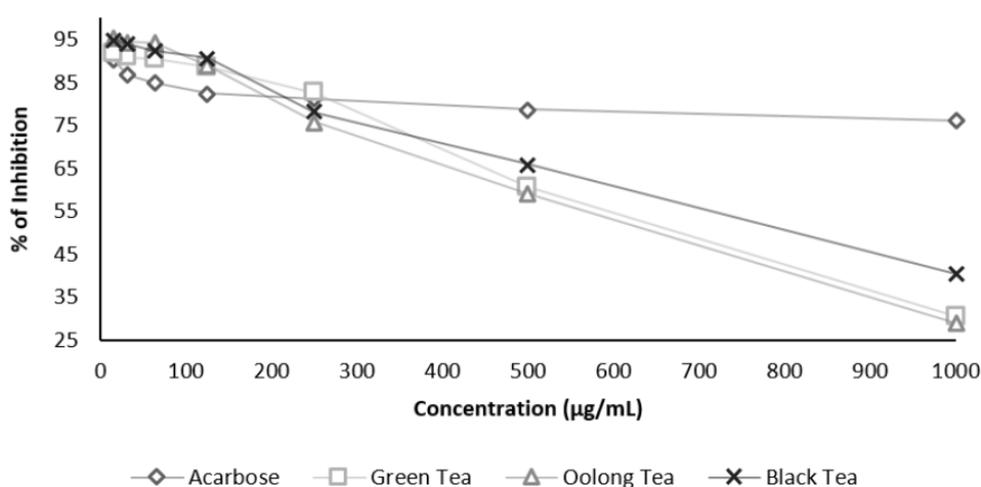


Fig. 2: Percentage inhibition of α -Glucosidase

Hence, this discovery highlights the significance of assessing the concentration in various tea types consumed to confirm their potency surpasses that of acarbose, and potentially making them viable alternatives to pharmaceutical inhibitors designed for glycosidase hydrolase enzymes.

Conclusion

In conclusion, different methods of processing of Sacha Inchi leaves showed distinct impacts on the phenolic components, free radical scavenging activity and inhibitory capabilities of green, oolong, and black Sacha Inchi tea. The green tea extract of Sacha Inchi showed high total phenolic content and total flavonoid content at concentration 500 μ g/mL with a value of 2.10 ± 0.01 μ g GAE/mL and 3.23 ± 0.20 μ g QE/mL respectively. Meanwhile, at a concentration of 500g/mL, Sacha Inchi black tea demonstrated strong antioxidant activity by DPPH with radical scavenging activity (%) (89.4

± 0.002). Besides while the inhibitory effects of green tea extract surpass those of acarbose at concentrations up to 250 μ g/mL (83%), the inhibitory effects of oolong and black tea extract was only stronger than acarbose up to 125 μ g/mL, indicating a relatively lower potency. However, green, oolong and black Sacha Inchi extract exhibit higher potency compared to acarbose at certain concentration and have the potential to serve as viable substitutes for pharmaceutical inhibitors targeting the glycosidase hydrolase enzymes. The findings of this study might support the notion that underutilized Sacha Inchi leaves are captivating sources of potential natural antioxidants.

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Conflict of Interest

All authors declare no conflict of interest.

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