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Physical Appearance Antioxidant Effect Alpha-amylase Inhibition and Alpha-glucosidase of Carissa Carandas Products

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This research aimed to examine tea production from the leaves and fruit of the Karanda (Carissa carandas) at different stages of development. The antioxidant activity of Carissa carandas leaves was determined by their total phenolic content, antioxidant capacity DPPH (2,2-diphenyl-1-picrylhydrazyl), and ferric-reducing capacity (FRAP). The experiment results were freeze-dried and ground into a powder. Young tea leaves had more antioxidants than older ones. Antioxidant capacity DPPH and ferric reduction ability were greater in Carissa carandas juice powder than in Carissa carandas powder. The amount of water extracted is greater. Using Carissa carandas powder, the leftover portion was substituted for tea leaves at weights of 0, 5, 10, 15, and 20 %. Color, total acid content, and total anthocyanin content were measured. The tea was a deeper shade of crimson. Substituting 20% of the fruit pulp for the Carissa carandas resulted in the greatest increase in redness and total acid content, including the total amount of anthocyanin. Superior to all other formulations to increase the total phenolic content, Carissa carandas development should be blended amongst the leaf portions. In sensory characteristics testing, it was determined that the highest acceptable substitution level on the 9-point Hedonic scale test was the 10% substitution level, with scores on appearance, color, odor, taste, texture, and preference that were distinct from those of other samples: 8.56, 8.89, 8.43, 8.21, 8.76, and 8.38.

Key words: Carissa Carandas, Karanda, Antioxidant, Alpha-Amylase, Alpha-Glucosidase.

1. INTRODUCTION

Most individuals now prioritize herbal tea use as a kind of health treatment. In addition, Thais have long consumed herbal tea to quench their thirst. Pohl et al. (2016) found that because each herbal tea includes a unique combination of chemicals in varying concentrations, it has several favorable effects on the body. Thus, regular consumption of herbal teas can promote health. Herbal teas are often brewed with hot water, herbs, fruits, seeds, or roots. Certain variants contain less antioxidants than teas brewed from tea leaves, but it is caffeine-, sugar-, and calorie-free and is available in various flavors. Certain teas have special qualities, such as antioxidant benefits.

Moreover, it possesses anti-alpha glucosidase enzyme activity, which reduces blood sugar levels and promotes health (Hidayati et al., 2015). Most herbal teas are caffeine-free, making them acceptable for the elderly and caffeine-sensitive individuals. In addition, herbal teas possess therapeutic effects, such as nerve stimulation. In addition to reducing cholesterol (Münstedt et al., 2009), anti-diabetes, high blood pressure, and cancer, antioxidants for the circulatory system can aid the digestive tract. The excretory system is typically functioning, and herbal tea aids in clearing the fat that attaches to the intestines and promoting heart health (Duygu et al., 2008). As a result, most people use herbal tea frequently.

Carissa carandas is a native Thai fruit. The scientific name for this plant is *Carissa carandas* Linn. It belongs to the Apocynaceae family (Aman et al., 2014). It is a family of berries. There are numerous Thai names, including Manao Ho, Thorn Khee Haad, and Thorn Daeng. The trunk is a tall, thorny shrub with white rubber on the fruit and leaves. The flowers are white and fragrant, forming a bouquet on the leaf axils. Towards the tip, it bears a little oval form. The fruit progressively transforms from white to pink, red, and dark purple when it ripens. The fruit's seeds are flat and arranged along its length. The leaves are singular, oval-shaped, and endowed with a small concavity. The leaf base is concave in the direction of the petiole. The back and abdomen are smooth, and the hue of the young leaves is pale red-green. Dark green mature leaves, thick leaves, and short petioles have a sour and astringent taste (Joshi et al., 2018), gradually decreasing as the fruit ripens and containing more anthocyanin.

The investigation of medicinal properties in pharmacological activities determined that mature fruit contained 1,022.9 mg ascorbic acid/100 ml juice (Arif et al., 2016) along with Total Phenolic Content 111.6 mg Gallic acid/100 g Sample and Total Anthocyanin Content 472.4 mg cyaniding-3-glucoside. The extraction of *Carissa carandas* fruit with chloroform and water yielded phenolic and flavone components. Therefore, DPPH, Superoxides, Hydroxyl, Hydrogen peroxide, and ABTS radicals are neutralized to the greatest extent. It can also

absorb iron ions. *Carissa carandas* can be exploited as a highly effective antioxidant source in the health food sector, as is evident (Verma et al., 2015). It affected ovarian cancer, Caov-3 cancer, and lung cancer cells. By extracting from three sections, namely leaf, raw fruit, and ripe fruit, it was discovered that chloroform-extracted leaf could effectively suppress the activity of Caov-3 cancer cells (Amaranath et al., 2021).

Carandinol, a triterpene, was also discovered in the leaves of the *Carissa carandas* tree. (Cytotoxicity) immunomodulatory, anti-glycation (Antiglycation), and antioxidant properties. In addition, the capacity to suppress enzyme activity in sterile conditions. This chemical was cytotoxic to all cancer cell types examined, including Hela, PC-3, and 3T3. It is highly effective against cervical cancer cells (Hela). Diabetes is a disease resulting from endocrine dysfunction. Abnormalities in the metabolism of carbs, fat, and lipoprotein not only result in excessively elevated blood sugar levels.

Nevertheless, also result in numerous problems, including hyperlipidemia, a disorder characterized by elevated insulin levels, high blood pressure, and atherosclerosis. According to ancient medical texts, *Carissa carandas*es possess anti-diabetic effects. Hence, an investigation into fruit extracts was done. It was discovered that extracts with methanol and ethyl acetate at 400 mg/kg could reduce blood glucose by 48 and 64.5%, respectively, compared to modern anti-diabetic medications. Hence, *Carissa carandas*es have the potential to be employed as anti-diabetes medications (Rao Dasari et al., 2020).

This study aims to identify physical traits. For instance, the antioxidant effect of Alpha-amylase inhibition and alpha-glucosidase of *Carissa carandas* goods obtained from creating *Carissa carandas* leaf tea and *Carissa carandas* at the ripe stage with the features of good color and flavor for customer information—entrepreneurs in the food and health supplement industries Those who study the use of therapeutic plants in food.

2. MATERIALS AND METHODS

1.1 Sample Collection and Sample Preparation

Obtain a leaf sample from the *Carissa carandas*. Beginning in July 2020 in the "Tha Maka District Kanchanaburi" by gathering leaves at various distances and locations: 1) Young leaves are harvested from the top of an apical leaf, which consists of one stem with two to three young leaves, 7 to 10 days after budding. 2) The middle leaf is obtained between the fifth pair of leaves from the top down that is neither too old nor too young. Gather two leaves. The age of the leaf 14 to 16 days after the shoot. 3) Old leaves gathered from the next pair of 6-7 leaves, arranged in threes, aged 21-25 days from the shoot's emergence.

In August 2020, samples of *Carissa carandas* fruit were collected from trees in Don Cha-Em Subdistrict, Tha Maka District, Kanchanaburi Province, by assembling all ripe dark purple fruits. After assembly, they are cleaned promptly.

Example preparation of *Carissa carandas* Leaf Tea Brings *Carissa carandas* leaves to be washed and cleaned with running tap water and pick out leaves of inferior quality. Then, immerse the leaves in tap water with 10 ppm chlorine for 10 minutes, rinse with running tap water for 5 minutes, drain, and cut into 0.5 cm width slices. Removed stems are utilized to produce tea leaves.

1.2 Preparation of Tea by the Method of Making Green Tea (Green Tea)

Non-fermented tea was made by heating *Carissa carandas* leaves for two minutes to deactivate the enzyme Polyphenol oxidase found in fresh *Carissa carandas* leaves. To prevent PPO from catalyzing the oxidation and polymerization of polyphenols in the leaves of *Carissa carandas*. Then, knead (rolling) the dough to break the cells. Dry in an oven at 60 degrees Celsius with hot air for one hour. The tea will be green to yellowish green in hue. And the ultimate moisture level is less than 10%.

Using a herb blender, thoroughly combine the tea leaves. And they are stored in a foil bag at -20 degrees Celsius till analysis.

Samples of *Carissa carandas* powder are prepared from overripe fruits. Takes *Carissa carandas*, washes them with running water, and sorts out old and imperfect fruit. The pulp was then placed in tap water containing ten parts per million of chlorine, steeped for ten minutes, rinsed with running tap water for an additional five minutes, drained, and extracted using a pulp extractor. Panasonic model MJ-DJ01S, freeze-dry all portions and finally combine using a herbal blender. The extracted water portion is known as Extract, whereas the waste portion is known as Residue. Before analysis, *Carissa carandas*' powder is stored in aluminum foil packets at -20 degrees Celsius.

Carissa carandas Mixing Combine the chosen *Carissa carandas* Leaves with the dehydrated and powdered *Carissa carandas* Fruits. Replace the tea leaves with Extract and residual powder of *Carissa carandas* at 0, 5, 10, 15, and 20 percent by weight, package in aluminum foil bags, and store at -20 °C until analysis.

1.3 Quality Testing of Tea Leaves and *Carissa Carandas* Powder

Color Measurement Measure the color of tea powder in the part of the tea leaves. *Carissa carandas* Powder and the mixed part With a Minolta colorimeter, CR 400 Japan in the CIE system, L* a* b* values are reported as L* illuminance values, ranging from 0 (black) to 100 (white) +a* (hugeness). red) -a* (greenish) +b* (yellowish) -b* (blueish)

1.4 Extract Preparation

Tea made from crushed *Carissa carandas* leaves, *Carissa carandas* Powder Extract, and Residue *Carissa carandas*, all five formulas (0, 5, 10, 15, and 20% by weight) were extracted by weighing 10 g dry samples. 90 cc of 40% water mixed with ethanol was used to soak 10 grams of prototypes for 1 day. The samples were run through

Whatman No. 4 filter sheets for filtration.

1.5 Determination of Total Phenolic Content

Using the Folin-Ciocateau assay, the authors of Sánchez-Rangel et al. (2013) determined "the content of the reducing component." Initially, 0.75 ml of the 10-fold diluted Folin-Ciocateau reagent and 100 ul of the ethanolic Extract were added to a test tube. The mixture was left at room temperature for five minutes after mixing. Then, 0.75 ml of 6% (w/v) sodium carbonate solution was added." After homogenization, the mixture was allowed to rest for 90 minutes at room temperature. At 725 nm, a spectrophotometer determined the total phenolic content. Ascorbic acid was utilized to plot the standard calibration curve at a concentration range of 0.02 to 0.1 mg/ml. The equivalent gallic acid (GAE) mg/g represents the total phenolic content."

1.6 Antioxidant Activity Test Free Radical-Scavenging Assay with DPPH

As reported previously, the antioxidant activity was performed with a few modest modifications. This was accomplished by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. A test tube containing 900 ul of DPPH in methanol solution (150 uM) was combined with 100 ul of each Extract and vigorously shaken. Following a 15-minute incubation period at room temperature in total darkness, the absorbance of each solution was measured at 517 nm. The ascorbic acid equivalent was employed to quantify the antioxidant activity."

1.7 Analysis of Ferric Acid Reducing Ability Ferric Reducing Antioxidant Potential (FRAP)

According to the protocol by Suktham et al. (2019), the FRAP assay was conducted. 2.25 ml of freshly prepared FRAP reagent was added to a test tube along with 75 ul of sample and 225 ul of distilled water. The mixture was utilized for the reaction at room temperature. The absorbance at 596 nm was measured with a spectrophotometer immediately after mixing and 30 minutes later. The antioxidant potential of the samples was determined using a calibration curve generated with FeSO4.7H2O at concentrations ranging from 400 to 2,000 uM.

1.8 Total Anthocyanin Content Assay

"Total anthocyanin content" was measured by "the pH-differential spectrophotometric method. Each sample was diluted with potassium chloride (0.025 M) at pH 1.0 and sodium acetate (0.4 M) at pH 4.5. They were allowed to equilibrate for 15 min before detection by spectrophotometer. The absorbance was measured at 520 nm and 700 nm". "The difference in the absorbance at differing pH values and wavelengths was calculated" as follows:

$$A = (A_{520nm} - A_{700nm})\ pH_{1.0} - (A_{520nm} - A_{700nm})\ pH_{4.5}$$

The concentration of total anthocyanin pigment was calculated as follows:

Total anthocyanin content (mg/ml) = (A x MW x DF x 1000) / ε x 1

Furthermore, "where MW is the molecular weight, DF is the dilution factor, ε is the molar absorptivity, and 1 is for 1 cm path length. The molecular weight (MW = 449.2 g mol-l) and the molar absorptivity (ε = 26,900 Lcm-1mol-l) of cyanidin-3-glucoside were used."

1.9 Inhibition assay for α-Glucosidase activity

α-Glucosidase inhibition assay

Accordingly, "appropriate dilutions of the extracts (0-200 µL) and 100 µL of α-glucosidase (EC 3.2.1.20) (0.5 mg/mL) in 0.1M phosphate buffer (pH 6.9) solution were incubated at 25°C for 10 min. Then, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside in 0.1 M phosphate buffer (pH 6.9) solution was added. The mixtures were incubated at 25°C for 5 min before the absorbance was read at 450 nm in the spectrophotometer (29). The reference sample included all other reagents and the enzyme except the test sample. The α-glucosidase inhibitory activity was expressed as percentage inhibition."

Inhibition (%) = [(Absref - Abssample)/Absref] × 100

1.10 Inhibition Assay for α-Amylase Activity

Appropriate dilutions (0-200 L) of the extracts and 500 L of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing porcine pancreatic -amylase (EC 3.2.1.1) (0.5 mg/mL) were incubated for 10 minutes at 25°C. Then, 500 L of a 1% starch solution in a sodium phosphate buffer of 0.02 M is added. After 10 minutes of incubation at 25°C, 1.0 mL of dinitro salicylic acid (DNSA) was added to the reaction mixture. The reaction was then stopped by incubation in a bath of boiling water for 5 minutes, then cooling to room temperature. The reaction mixture was then diluted with 10 mL of distilled water, and absorbance at 540 nm was determined using a spectrophotometer (28). "Except for the test sample, the reference sample contained all other reagents and the enzyme." The percentage of inhibition was used to express the -amylase inhibitory activity.

Absref = absorbance of the reference; Abssample = absorbance of the test sample.

3. Experimental Design and Statistical Analysis

The CRD experiment was designed with three levels of leaf senescence, obtained using the green tea technique as the sole study component. Then assess the quality based on the color value information derived from antioxidant properties. Using a pre-packaged application, the variance and difference of means were analyzed (Duncan multiple range test method).

The CRD experimental design was utilized to investigate the preparation of freeze-dried *Carissa carandas* fruit powder. One study factor consisted of two extraction levels based on the color data: extracted and Residue. The variance and mean differences were analyzed (Duncan multiple range test method) using a pre-packaged program for antioxidant characteristics.

Examine the proportion of organic tea leaves to *Carissa carandas* powder. Planned for the CRD experiment were a single variable, the leaf-to-fruit ratio—five substitution levels with weight percentages of 0, 5, 10, 15, and 20 percent. Select from the data on color values. The variance and difference of means were examined (Duncan multiple range test method) using a pre-packaged application to determine antioxidant qualities.

4. RESULTS

Effects of preparation of *Carissa carandas* leaves on physical and chemical characteristics of tea leaves. However, in every table of results, "data were presented as mean ±, standard deviation (S.D.), and different superscripts within a column significantly different ($p<0.05$)."

The statistical analysis found that the %Yield %Moisture, and Water activity of the *Carissa carandas* leave with different maturity levels. The different preparation methods resulted in the results shown in Table 1. The combined effect of the preparation and maturity of the *Carissa carandas* leaves on the color values was statistically significant ($p<0.05$), as follows: Table 2 also affects the number of phenolic compounds. And the antioxidant capacity, as shown in Table 3, respectively.

Table 1: % Yield %Moisture and Water activity of karanda leaf tea prepared by green tea making method

Leaf stage	%yield	$\%Moisture_{ns}$	Water Activity
leaflet	15.13 ± 0.25^{b}	3.68 ± 0.16	0.44 ± 0.04^{b}
middle leaf	15.17 ± 0.46^{ab}	3.70 ± 0.12	0.51 ± 0.03^{a}
basal leaf	16.20 ± 0.20^{a}	3.59 ± 0.13	0.47 ± 0.04^{ab}

Table 2: Effect of leaf stage and color measurement (CIE L* a* b*) of karanda leaf tea prepared by green tea making method.

Leaf stage	L*	a*	b*
leaflet	40.41 ± 0.37^{c}	1.36 ± 0.37^{a}	5.45 ± 0.28^{c}
middle leaf	46.51 ± 0.34^{b}	-2.76 ± 0.29^{b}	13.74 ± 0.16^{a}
basal leaf	48.40 ± 0.67^{a}	-3.63 ± 0.06^{c}	12.64 ± 0.25^{b}

Various maturation phases of Carissa caranda leaves can impart distinct hues to the tea. Young-leaf tea is a reddish-brown color. The tea is prepared from mature and middle-aged leaves. It will be green-yellow. Tea from mature leaves was darker than from young or intermediate leaves. The colorimeter results indicated that the L* and lightness values of tea prepared from young leaves were light. The yellowness b* was lower, while the redness a* was greater than in previous phases. The results indicated that the color of *Carissa carandas* leaves depends on the kind and quantity of pigment. Particularly chlorophyll, which is a vital component of the leaves of *Carissa carandas*. The chlorophyll concentration is greatest in mature leaves and declines in wilted leaves (Yu et al., 2019). Young and middle leaves Moreover, when chlorophyll is heated, it transforms into Pheophytin, causing the leaf color to become a dark green or olive green (Zhang et al., 2019).

Table 3: Effect of leaf stage and tea production methods towards total phenolic content and antioxidant activity

Leaf stage	Total phenolic content (mg GAE/g dry sample)	DPPH (mg AAE/g)	FRAP (umolFeSO4/g)
leaflet	29.73 ± 0.67^{c}	65.04 ± 0.49^{c}	343.82 ± 1.15^{c}
middle leaf	52.19 ± 0.54^{b}	70.67 ± 0.47^{b}	374.94 ± 2.56^{b}
basal leaf	55.69 ± 0.21^{a}	80.19 ± 0.91^{a}	402.30 ± 0.69^{a}

Quantities and types of phenolic chemicals in *Carissa carandas* leaves depend on leaf age and development, leaf location, collection time, and growing season (Hegde et al., 2010). In addition, the processing procedure altered the kind and number of active components, which directly affected the antioxidant activity of phenolic compounds extracted from tea leaves. The key chemicals in *Carissa carandas* leaves are flavonoid molecules with antioxidant properties. Table 3 demonstrates that tea made from leaflet leaves contained fewer total phenylic chemicals than tea manufactured from middle and basal leaves. DPPH antioxidant and ferric acid reducing capacity. It also depends on the tea's ingredient type and overall phenolic content. Tea leaves made from mature leaves had more antioxidant capacity, DPPH, and ferric acid-reducing capacity than those made from medium and young leaves due to the accumulation of nutrients and minerals in mature leaves. Then tea leaves with higher chlorophyll and phenolic component contents. Consequently, in *Carissa carandas*, mature leaves are chosen as the tea's constituents.

- Along with *Carissa carandas* Leaves, prepare *Carissa carandas* Powder as a basic material for preparing tea. Carandas of Carissa that are ripe has been prepared. The pulp and water of the dark purple fruits were freeze-dried after extraction. The yield percentage, moisture content, and water activity were examined according to Table 4.

Table 4: % Yield %Moisture and Water activity of Caranda Powder

Karanda Powder	%yield	%Moisture	Water Activity
Extract	8.07 ± 0.05^{b}	10.53 ± 0.78^{a}	0.57 ± 0.01^{a}
Residue	9.57 ± 0.05^{a}	4.36 ± 0.34^{b}	0.16 ± 0.01^{b}

The pulp was extracted by centrifuging and pressing off the juice, yielding two extracts: Extract and Residue. The investigation revealed that the %yield of Residue was

greater than that of Extract because Residue consisted of fruit pulp, peel, and seed, which comprised pulp. Thus, coarser fibers are a component of cellulose and hemicellulose and plant cell walls. Thus, there is less moisture than in the fruit extract, which contains water and a fine texture. As anything undergoes freeze-drying, its weight decreases. In addition, the two ways of raw material processing resulted in distinct acid contents. The extract powder was more acidic. Hence, it absorbs moisture faster, resulting in greater %Moisture and Water Activity than residual samples (see Table 5).

Table 5: Color measurement (CIE L* a* b*) of karanda powder

Karanda Powder	L*	a^*_{ns}	b*
Extract	6.80±0.46[b]	31.00±0.75	10.47±0.35[b]
Residue	8.67±0.55[a]	32.24±0.90	14.91±0.50[a]

Depending on the anthocyanin content of the fruit, the brightness of the Karanda's power varies. Depending on the quantity of the material detected, it produced a purple hue. The brightness of the Extract was less than that of the Residue. The experimental outcomes corresponded to Table 6. The Extract contained more anthocyanin than the Residual. The redness was identical.

Table 6: Total phenolic Total Anthocyanin content and antioxidant activity of karanda powder

Karanda Powder	Total phenolic content (mg GAE/g dry sample)	Total Anthocyanin	DPPH (mg AAE/g)	FRAP (umolFeSO4/g) $_{ns}$
Extract	53.95±0.95[b]	16.92±0.23[a]	31.24±0.40[b]	9.55±0.48
Residue	67.13±0.76[a]	12.85±0.80[b]	37.41±0.71[a]	8.52±0.38

According to Table 6, the total phenolic content of Residue was larger than that of Extract because several fruit ingredients, such as rind, flesh, seed coat, and seed, caused the seeds to explode when blades crushed them. In addition, the release of phenolic compounds from the seeds resulted in the Residue possessing a higher Total phenolic content and DPPH antioxidant capacity than the Extract, which consisted primarily of water from meat. The two components were determined to be identical regarding their ability to decrease ferric acid. When examining anti-diabetes activity by testing the inhibition of Alpha-amylase and Alpha-glucosidase enzymes, Figures 1 and 2, it was discovered that *Carissa carandas* Extract from Extract and Residue at a concentration of 100 mg/ml in distilled water inhibited the enzyme activity by 98.70% and 82.53%, respectively.

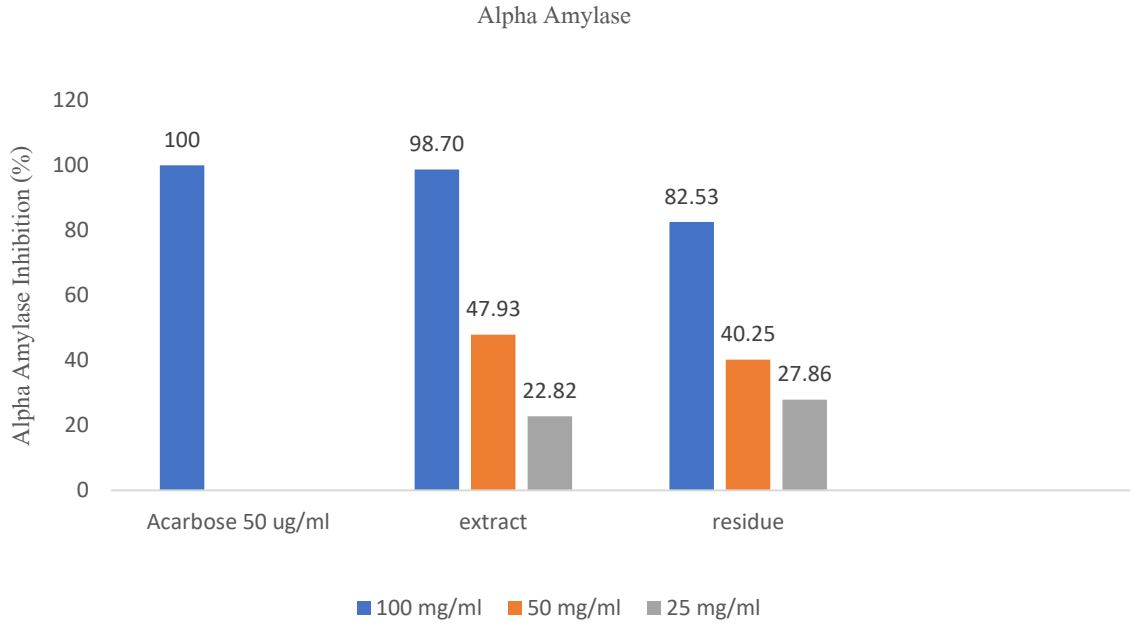

Figure 1. Alpha-amylase inhibition of karanda powder

Compared to the anti-diabetic medicine Acarbose, the Extract of ripe *Carissa carandas* was more effective. At various doses, freeze-dried polysaccharides were able to suppress the activity of the Alpha amylase enzyme. Also, there is no concentration limit because *Carissa carandas* powder is a medicinal herb that can be consumed safely. It can be consumed to reduce the digestion of glucose and starch. Reduce the rate of glucose absorption. Hence, the stomach and intestines can be employed as a food ingredient or consumed in another manner to reduce blood sugar levels. The experimental outcomes correspond to Figure 2. Both halves of the *Carissa carandas* extract *Carissa carandas* can block alpha-glucosidase. Both types of enzymes exist in the human body. Moreover, the stomach and small intestine can convert starch into individual sugar molecules.

Moreover, several cells will absorb the sugar into the bloodstream. Using *Carissa carandas* extract to suppress the activity of both enzymes prevents the enzyme from binding to the substrate, including starch, resulting in an incomplete enzyme reaction. Thus, it does not cause glucose and is the identical method by which anti-diabetic medications work. In contrast, the use of Karanda powder involves the utilization of plant extracts. Thus, the inhibitor's concentration and impact are not equivalent to the medications. As a result, it is more appropriate to consume to control and improve the body's immunity than to treat disease.

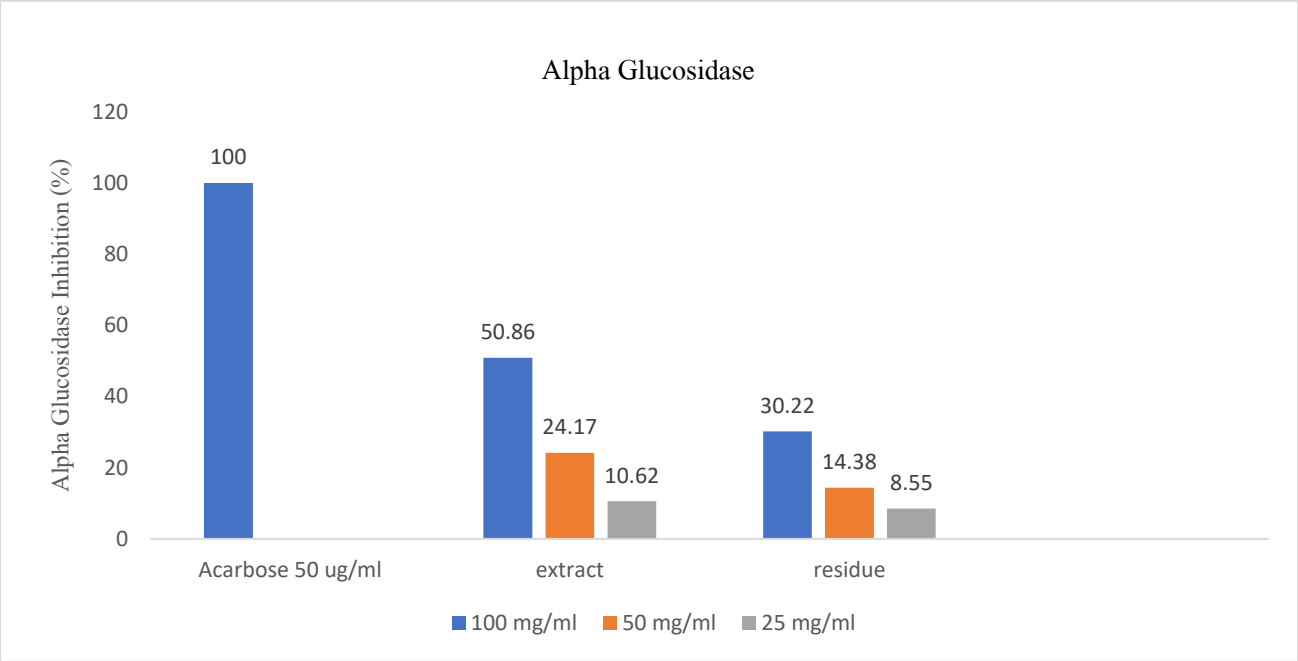

Figure 2. Alpha-glucosidase inhibition of karanda powder

It involves selecting Karanda powder as an ingredient in tea alongside tea leaves. Hence, the Extracted portion is chosen due to its higher yield and lower moisture and water activity percentages. Reduced moisture will help preserve the tea's quality. During storage, high humidity makes the product susceptible to deterioration by mold. In addition, Residue has a greater total phenolic content and DPPH antioxidant capacity than Extract. Both the leaves and the fruit of *Carissa carandas* contain free radicals.

The optimal ratio of tea leaves and freeze-dried powder on the physical and chemical properties of *Carissa carandas* is determined by this study.

Mixing (formulate) (formulate) Tea leaves from mature *Carissa carandas* leaves and *Carissa carandas* freeze-dried powder from fruit leftovers at five different ratios influenced %Moisture and Water Activity values (Table 7), as well as color quality (Table 8).

It was discovered that when the proportion of *Carissa carandas* powder grew, the percentage of moisture and water activity increased, respectively. The ratio of leaves to fruits was 90:10 and 95:5. The levels of %Moisture and Water Activity were identical to those of the control samples. While the 85:15 and 80:20 ratios had higher levels of %Moisture than other samples, Due to the very acidic nature of the *Carissa carandas* fruit, it can absorb and retain moisture in the powder at all times. This causes the humidity to be higher than the *Carissa carandas*' leaves.

Table 7: The ratio of leaf to the fruit of Carissa carandas on the physical quality of ready-to-brew tea

Karanda leaf tea: Karanda Powder	%Moisture	Water Activity $_{ns}$
100: 0	3.59±0.13^{c}	0.47±0.04
95: 5	3.68±0.15^{c}	0.48±0.05
90: 10	3.72±0.23^{c}	0.48±0.04
85: 15	4.15±0.26^{b}	0.50±0.05
80: 20	4.57±0.25^{a}	0.51±0.05

Table 8: The ratio of *Carissa carandas* leaf to *Carissa carandas* fruit depends on the color quality of ready-to-brew tea powder

Karanda leaf tea: Karanda Powder	L*	a*	b*$_{ns}$
100: 0	48.40±0.67^{a}	-3.63±0.06^{e}	12.64±0.25
95: 5	45.83±0.57^{b}	1.58±0.22^{d}	12.62±0.22
90: 10	42.42±0.36^{c}	2.65±0.41^{c}	12.66±0.32
85: 15	40.56±0.35^{d}	3.73±0.17^{b}	12.81±0.56
80: 20	39.41±0.37^{e}	6.41±0.23^{a}	12.90±0.11

From Table 8, when the proportion of powdered *Carissa carandas* led to a decrease in the tea's brightness. When the proportion of *Carissa carandas* powder grew, so did the redness value. Powdered *Carissa carandas* are made from ripe fruit that has been dried using the free fresh process. In addition, heat does not destroy anthocyanin during the drying process, allowing the raw material to be ground into a powder with a permanent red hue. In addition, the increased amount of powder led to an increase in the concentration of anthocyanin (Table 9), which played a crucial part in the red color of the blended tea.

Table 9: The ratio of leaves to the fruit of *Carissa carandas* on the chemical quality of ready-to-brew tea

Karanda leaf tea: Karanda Powder	Total phenolic content (mg GAE/g dry sample)	Total Anthocyanin (mg/L)	DPPH (mg AAE/g)	FRAP (umolFeSO4/g)
100: 0	55.76±0.06^{c}	0.00±0.00^{e}	80.35±0.47^{a}	402.73±0.70^{a}
95: 5	56.24±0.27^{c}	2.72±0.50^{d}	79.93±0.34^{a}	384.53±4.96^{b}
90: 10	57.62±0.44^{b}	4.52±0.29^{c}	77.43±0.38^{b}	375.88±1.16^{c}
85: 15	58.37±0.34^{a}	6.44±0.39^{b}	76.30±0.72^{c}	372.18±0.60^{c}
80: 20	58.41±0.52^{a}	8.37±0.33^{a}	69.47±0.36^{d}	361.93±0.47^{d}

In blended tea, the weight ratio of tea leaves to *Carissa carandas* fruit is 80:20. The ratios of total phenolic content and anthocyanin were greater than all others. Both tea leaves and *Carissa carandas* fruit contain a high concentration of essential chemicals. *Carissa carandas* contains more anthocyanins in its ripe fruit than in its leaves. Thus, the addition of *Carissa carandas* powder from the ripe fruit section led to an increase in overall anthocyanin concentration.

Antioxidant capability DPPH and the ability to reduce ferric acid Found in the older leaves of the tea plant by decreasing the amount of *Carissa carandas* leaves in the tea, the antioxidant capacity of DPPH, and the ability of ferric acid are diminished. The outcomes are shown in Table 10.

Table 10: The ratio of leaf to the fruit of Carissa carandas on the physical and chemical quality of ready-to-brew tea

Karanda leaf tea: Karanda Powder	% Inhibition of Alpha amylase enzyme	% Inhibition of Alpha-glucosidase enzyme
100: 0	97.36±0.25^{a}	51.03±0.77^{a}
95: 5	96.11±1.18^{bc}	50.39±0.24^{ab}
90: 10	97.14±0.26^{ab}	49.91±0.21^{bc}
85: 15	96.32±0.12^{abc}	49.67±0.38^{bc}
80: 20	95.54±0.28^{c}	49.14±0.77^{c}

Different proportions of Alpha amylase enzyme and Alpha glucosidase enzyme activity are inhibited by blending varying concentrations of tea. Tea with a high proportion of *Carissa carandas* leaves inhibits Alpha amylase enzyme and Alpha-glucosidase activity more effectively. Few tea leaves are present. The entire portion of the *Carissa carandas* is visible. Moreover, *Carissa carandas* fruit can reduce the activity of both enzymes. Hence, *Carissa carandas* have great promise for use in tea preparations—or powdered beverages made with hot water to boost immune, antioxidant, and anti-diabetes properties.

From the sensory characteristics test conducted by brewing one sachet of assorted tea weighing 3 grams with 200 grams of hot water at 90 degrees Celsius, as shown in Table 11, the 90:10 ratio of mixed tea was the most acceptable in terms of appearance, color, aroma, flavor, and astringency. And preference in general. The tea's look is transparent and pink—the aroma of tea leaves blended with the fragrance of delicate fruits. *Carissa carandas* Powder, which is mildly astringent and does not leave a bitter aftertaste on the tongue, has the potential to be developed as a nutritional supplement and health drink. Featuring the ability to combine with other culinary items.

Table 11: The ratio of leaves to the fruit of Carissa carandas on sensory properties of ready-to-brew tea

	sensory features					
Karanda leaf tea: Karanda Powder	Appearance	color	smell	taste	astringency	overall preference
100: 0	7.11±0.42^{b}	6.89±0.42^{d}	7.00±0.43^{d}	7.22±0.69^{c}	6.67±0.38^{b}	6.67±0.51^{d}
95: 5	7.22±0.76^{b}	6.57±0.50^{d}	7.43±0.50^{c}	7.33±0.50^{b}	6.62±0.51^{c}	7.52±0.51^{b}
90: 10	8.00±0.24^{a}	8.67±0.42^{a}	8.24±0.61^{a}	7.76±0.91^{a}	7.95±0.62^{a}	8.90±0.50^{a}
85: 15	6.22±0.73^{b}	8.05±0.70^{b}	8.33±1.00^{b}	6.67±0.50^{c}	7.29±0.49^{b}	7.29±0.43^{b}
80: 20	6.33±0.51^{b}	7.48±0.64^{c}	7.57±0.51^{c}	6.38±0.69^{c}	7.14±0.50^{b}	7.00±0.24^{c}

5. CONCLUSION AND DISCUSSION

Carissa carandas formed from leaves of varying maturation have distinct hues and antioxidant capabilities. Tea made from mature leaves was darker and yellower than young and middle-aged leaves. In terms of its antioxidant properties, tea made from mature leaves was found to have the highest antioxidant activity, as measured by the total amount of "phenolic compounds, antioxidant capacity," and the ability to reduce ferric acid the most when compared to tea made from young and medium-aged leaves.

Carissa carandas Both the Extract and Residue of freeze-dried powder possess antioxidant potential. They can inhibit alpha-amylase activity. and alpha-glucosidase the powdered Residue of *Carissa carandas* comprises the complete amount of "phenolic chemicals." In addition, the "antioxidant capacity" and the ability to decrease ferric acid were greater than those of the Extract produced using the extract method. It was discovered that the Extract had a greater total anthocyanin content. Moreover, the red

color was more prominent than the Residue. *Carissa carandas* powder made as an extract included a higher "moisture content" and Aw than the Residue, making it easier to create sticky lumps. While using the produced residue of *Carissa carandas* powder to raise the antioxidant content of tea leaves, it was discovered that a 10 percent increase enhanced the flavor of the tea. There was an increase in sour and sweet, whereas astringency decreased. In addition, people acknowledge that *Carissa carandas* can be utilized to create a healthy tea product: blood sugar-lowering antioxidants and anti-diabetes agents. This study has contributed to the corpus of knowledge with fresh insights, as no prior research has examined this behavior about tea and *Carissa carandas*. This research has added fresh insights to the literature concerning using *Carissa carandas* for tea. In addition, companies can use the findings of this study to improve the color and flavor of their tea by working harder. With the help of *Carissa carandas*, these discoveries can be employed in a major way to improve the production of various tea varieties. Hence, this study contributes to the corpus of knowledge. Nonetheless, it is advised that future researchers continue to work on this topic and develop more dimensions and trials that could provide a better method for producing tea formulas with improved flavor and quality.

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