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# Tyrosinase Inhibitory Activity of Combined *Camellia sinensis* (L.) Kuntze and *Oryza sativa* (L.) Extract Incorporated Serum

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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***Original Research Article***

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## ABSTRACT

**Aims:** The efficacy of combined *Camellia sinensis* (L.) Kuntze (green tea) and *Oryza sativa* (rice bran) extract in inhibiting the activity of tyrosinase was evaluated.
**Methodology:** Phytochemical analysis of the extracts was performed to detect various contituents using standard techniques, including LC-MS and GC-MS. Water- and oil-based serum were formulated with an optimum amount of combined *Camellia sinensis* and *Oryza sativa* extract. Tyrosinase assay of both formulas was determined with the presence of DOPA as oxidase activity.
**Results:** Anthocyanins and fatty acids were found in rice bran, whereas the green tea sample contained mainly catechins including GC, EGC, EC, EGCG, and ECG. The combination of the two extracts of equal amount (w_w) at 100 ppm achieved the optimum percentage of tyrosinase inhibitory activity (56.76% ± 0.15). Tyrosinase inhibitory activity of two serum products (water- and oil-based) containing 1% of combined *Camellia sinensis* and *Oryza sativa* extract (ratio of 5:5) were 18.7% ± 0.38 and 22.5% ± 0.41, respectively.
**Conclusion:** The combination of *Camellia sinensis* and *Oryza sativa* extract with the ratio of 5:5 (w_w) achieved the highest percentage of tyrosinase inhibitory activity. The catechins in green tea

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extract protecting against lipid oxidation of fatty acids of rice bran oil might play a key role in enhancing tyrosinase inhibitory activity.

*Keywords: Camilla sinensis; Oryza sativa; LC-MS; GC-MS; tyrosinase inhibitor; serum.*

## 1. INTRODUCTION

Green tea obtaining from the tea plant *Camellia sinensis* (L.) Kuntze (*C. sinensis*) is a Camellia genus member and belongs to the Theaceae family. Green tea has been commonly used as medicinal plant throughout the ages in China, India, Japan, Vietnam and many other Asian countries. The tea plant has an abundance of beneficial compounds. It includes almost 4,000 metabolites, more than a third of which belongs to the polyphenol group. Green tea consists of tannins, caffeine, proteins, essential oils, yeasts, pigments, pectin, vitamins, minerals, and organic acids. In addition, green tea is extremely rich in catechins including epigallocatechin gallate (EG), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), gallocatechin (GC), catechin (C), epicatechin (EC), gallate-epicatechin (ECG), and epigallocatechin (EGC). It has been reported that green tea has numerous applications in cosmetics such as hydrating the skin and preventing wrinkles, and in toothpaste for its antibacterial properties. It is well known that catechins in green tea, especially EGCG, possess not only antioxidative property but also tyrosinase inhibitory activity, which is known to govern the rate-limiting phase of melanin synthesis [1-4].

*Oryza sativa* L. (*O. sativa*), which is cultivated mostly in the tropics and subtropics, is the oldest crop in the world. The rice milling process produces rice bran, which accounts for around 10% of the rice's bulk. Rice bran is composed of the endosperm, germ, and embryo of the grain, as well as fragments of the grain. Rice bran has a brilliant yellow hue and a distinct odor. Rice bran contains proteins, lipids, carbohydrates, fibre, vitamins and minerals. Rice bran possesses about one hundred physiologically active compounds, including γ-oryzanol, ferulic acid, tocotrienol, tocopherol, octacosanol, squalene, γ-amino butyric acid, and phytic acid. Rice bran oil is used in cosmetics and medicines for the treatment of acne, oily skin, rashes, and the prevention and treatment of vitamin deficiency, cardiovascular disease, and macular degeneration. It has been reported that metabolites compounds in rice bran inhibit tyrosinase activity and melanin formation by different mechanisms [5-7].

Tyrosinase is a multi-copper enzyme present in several organisms. Tyrosinase produces melanin by oxidizing an amino acid called tyrosine. This enzyme is found mostly in melanocytes which is the pigment-producing cells of the body. Melanin is present in two basic forms in human skin: black/brown eumelanin and yellow/red pheomelanin, which are both synthesized from tyrosine via a shared biochemical pathway. Copper-dependent tyrosinase catalyzes the initial rate-limiting step in the conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and is regulated by tyrosinase-related protein (TRP-1 and TRP-2). Tyrosinase contributes to the regulation and control of melanin synthesis. Tyrosinase may either directly oxidize 5,6-dihydroxyindole-2-carboxylic acid (DHICA) to eumelanin or indirectly oxidize DHICA and 5,6-dihydroxyindole (DHI) through the production of o-dopaquinone. As a result of the attack of glutathione or L-cysteine on o-dopaquinone, glutathione-dopa or cysteinyl-dopa adducts are produced, which eventually transform into pheomelanin [8]. When exposed to ultraviolet radiation, the formation of melanocytes and skin pigmentation will protect the skin from UV damage as well as the formation of skin photo-carcinogenesis. Since the inhibition of tyrosinase prevents melanin accumulation in skin, tyrosinase inhibitors have been targeted as skin-whitening agents in cosmetics [9-11].

## 2. MATERIALS AND METHODS

### 2.1 Collection and Preparation of Plant Materials

*C. sinensis* leaves were harvested and washed several times with water to remove impurities. The green tea leaves were immediately inactivated enzyme by steaming at 100°C for 3 minutes and then dried at 70°C to reach a moisture content of 3-5%. Finally, they were ground into powder by a mechanic grinder and stored in an airtight bag in a dry place, protected from light until use. Meanwhile, rice bran, taken from the rice mill, was sieved to remove impurities before being dried at 110°C for 3 hours to reach a moisture content of 5-6% suitable for extraction. Rice bran powder was stored in sealed, mold-proof containers.

## 2.2 Preparation of Plant Extraction

Powder of *C. sinensis* leaves was extracted by ethanol 70% using the Soxhlet apparatus. After the filtration with No. 2 Whatman filter paper, the extract was concentrated by rotary evaporation and was further freeze-dried under a vacuum and then stored in refrigerator for subsequent use. Meanwhile, rice bran was extracted by Ultrasound Assisted Extraction (UAE) with the frequency of 20 kHz in ethanol 50% for 40 minutes at 40°C with a ratio of raw materials and solvents of 1:10. The extract was filtered and centrifuged at 4000 rpm for 5 minutes. The extract was then concentrated by rotary evaporation and stored in a refrigerator for later use.

## 2.3 Phytochemical Screening

Phytochemical analysis of the extracts was performed for the detection of various contituents using standard techniques, including LC-MS and GC-MS.

## 2.4 Tyrosinase Assay

The extracts of green tea and rice bran were combined in the following proportions: 10:0, 8:2, 6:4, 5:5, 4:6, 2:8, and 0:10. In addition to L-DOPA, the tyrosinase inhibitory action of the combination was evaluated using L-tyrosine as a substrate. The L-tyrosine test included a total of four wells, which were labelled as follows: A (control), B (blank control), C (sample), and D (blank sample). Samples consisting of seven different ratios of a mixture of green tea and rice bran were dissolved with 5% DMSO in a phosphate buffer of pH 6.8 (Table 1).

After 40 minutes of incubation at 25$^{o}$C, the absorbance at 475 nm was applied to measure the enzyme activity, and the percent tyrosinase inhibition was computed using the following formula:

$$\text{Tyrosinase inhibition } (\%) = \frac{(A-B)-(C-D)}{A-B} \times 100$$

Where A is the absorbance of the control with enzyme, B is the absorbance of the control without the enzyme, C is the absorbance of the test sample with the enzyme, and D is the absorbance of the test sample without the enzyme.

## 2.5 Serum Formulation

### 2.5.1 Water-based serum

The product was prepared in three phases using ingredients shown in Table 2.

For phase A, xanthan gum was added into distilled water at room temperature and stirred gently to avoid foaming. Next, allantoin and other ingredients were dissolved in a beaker warmed by a water bath at 70$^{o}$C, creating phase B. Preparation of the water-based serum continued as phase A and B were combined with continuous agitation and finished by the addition of a mixture of *O. sativa* and *C. sinensis* extract before distilled water was added to make up 100 g of formula.

**Table 1. The procedure of tyrosinase assay**

| | Control (A) | Blank control (B) | Sample (C) | Blank sample (D) |
|---|---|---|---|---|
| Phosphate buffer (pH 6.8) (µL) | 120 | 160 | 80 | 120 |
| Sample (µL) | | | 40 | 40 |
| Tyrosinase 46 U/mL (µL) | 40 | | 40 | |
| | | **Incubate for 10 min at 25°C** | | |
| L-DOPA 2.5 mM (µL) | 40 | 40 | 40 | 40 |

**Table 2. Formulation of water-based serum**

| Phase | Ingredient | Percentage (%) |
|---|---|---|
| A | Xanthan gum | 2% |
| B | Allantoin | 0.5% |
| | Vitamin E | 1.5% |
| | Provitamin B5 | 0.5% |
| | Aloe vera | 1.5% |
| | Glycerin | 0.5% |
| | Amodimethicone | 2% |
| C | Tween 80 | 1% |
| | A mixture of *O. sativa* and *C. sinensis* extract | 1% |
| | Citric acid | Adjust pH ~ 5.5 |
| | Water | ~ 90% |

**Table 3. Formulation of oil-based serum**

| Phase | Ingredient | Percentage (%) |
|---|---|---|
| A | Xanthan gum | 2% |
| B | Allantoin | 0.5% |
| | Vitamin E | 0.5% |
| | Provitamin B5 | 0.5% |
| | Aloe vera | 1.5% |
| | Glycerin | 0.5% |
| | Amodimethicone | 2% |
| | Cetyl alcohol | 8% |
| | Coconut oil | 10% |
| | Jojoba oil | 10% |
| C | Tween 80 | 1% |
| | A mixture of *O. sativa* and *C. sinensis* extract | 1% |
| | Citric acid | Adjust pH ~ 5.5 |
| | Water | ~ 62% |

### 2.5.2 Oil-based Serum

The product was prepared in three phases using ingredients presented in Table 3.

The preparation of oil-based serum was similar to that of water-based serum. However, ingredients in phase B were dissolved in a beaker heated by a water bath at 100$^{o}$C.

## 2.6 Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed in terms of Mean ± Standard Error of Mean (SEM). Statistical analysis was performed by SPSS and analysis of variance (ANOVA) with the level of significance $P$ = .05.

## 3. RESULTS AND DISCUSSION

## 3.1 Phytochemical Screening of Green Tea Extract

As can be seen from Fig. 1, the substances GC (peak #5), EGC (peak #6), EC (peak #7), EGCG (peak #8), and ECG (peak #9) have been identified as catechins based on the retention time, peak area and the standard substances of the obtained signals. In general, catechins, the major phenolic compounds, were found abundant in green tea with a relative peak area for chromatograms of 98.9%.

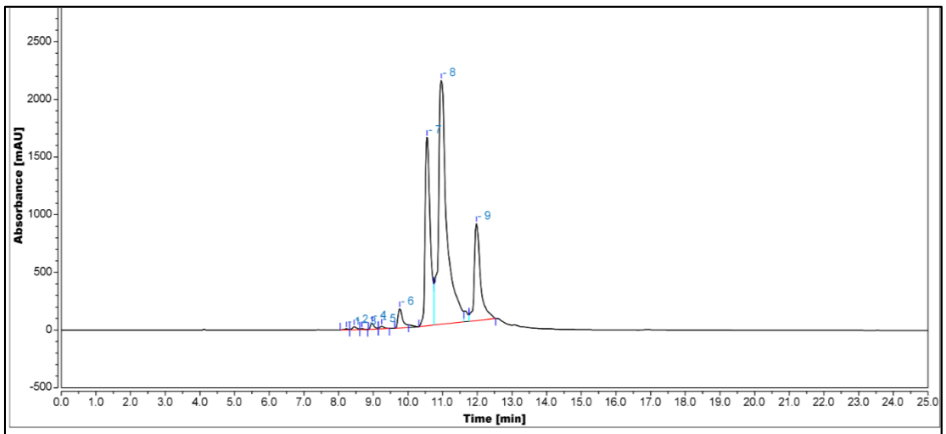
## 3.2 Phytochemical Screening of Rice Bran Extract

As can be seen from Fig. 2 and Table 4, linoleic acid was accounted for the most percentage of non-polar compounds in rice bran (79.79% ± 0.22), and it was also targeted as a possible tyrosinase inhibitor. Meanwhile, three substances belonging to anthocyanins were found in rice bran by LC-MS: Cyanidin-3-O-galactoside, coniferin, and malvidin; however, the quantities were not statistically significant.

## 3.3 Tyrosinase Assay Results

The ratios of *C. sinensis* and *O. sativa* in the combined samples were made in the following proportions: 10:0, 8:2, 6:4, 5:5, 4:6, 2:8, and 0:10. Their ability to inhibit tyrosinase enzyme was examined (Table 5).

As can be seen from Table 5, there was a significant difference in tyrosinase inhibitory activity among seven different ratios of *C. sinensis* and *O. sativa* extract. However, the tyrosinase inhibitory activity of green tea was much stronger than that of rice bran (52.29% ± 0.33 versus 5.6% ± 0.11). It is well known that catechins found in green tea have been proven to be the key components responsible for inhibiting tyrosinase activity whereas anthocyanins and fatty acids in rice bran exhibit low tyrosinase inhibitory activity. It has been reported that anthocyanins may inhibit tyrosinase by substituting a substrate that competitively binds tyrosinase, hence lowering product synthesis [12]. In addition, it could chelate some metal ions, allowing them to operate at the active site [13]. It has been observed that anthocyanins may successfully reduce $Cu^{2+}$ ions. On the other hand, linoleic acid and palmitic acid were found to modulate the regulation of tyrosinase proteolysis. Other research revealed that unsaturated fatty acids like oleic acid or linoleic acid had the potential to inhibit melanin formation. In contrast, saturated fatty acids like stearic acid or palmitic acid promote melanogenesis [14]. As can be seen from the GC-MS result (Fig. 2 and Table 4), the percentage of linoleic acid (79.79% ± 0.22) in the rice bran sample was much larger than that of palmitic acid (2.41% ± 0.15), suggesting that linoleic acid in rice bran might be the key contributor to the inhibition of tyrosinase activity. However, fatty acids are not regarded as “true tyrosinase inhibitors”. Fatty acids can modulate melanin production without changing melanogenesis protein expression. The mechanism of this action is that tyrosinase is selectively targeted by fatty acids and is based on enzymatic degradation in a proteasome-dependent physiological mechanism, resulting in changes in the protein tyrosinase content of hyperactive melanocytes without changes in the TRP-1 or TRP-2 proteins [15-17].




**Integration Results**

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|---|---|---|---|---|---|---|---|
| 1 | | 8.223 | 1.085 | 8.373 | 0.10 | 0.17 | n.a. |
| 2 | | 8.453 | 3.737 | 25.555 | 0.34 | 0.52 | n.a. |
| 3 | | 8.670 | 0.855 | 6.634 | 0.08 | 0.14 | n.a. |
| 4 | | 8.967 | 6.570 | 52.101 | 0.60 | 1.07 | n.a. |
| 5 | | 9.240 | 3.187 | 22.606 | 0.29 | 0.46 | n.a. |
| 6 | | 9.763 | 26.517 | 168.605 | 2.42 | 3.46 | n.a. |
| 7 | | 10.550 | 282.004 | 1633.972 | 25.71 | 33.52 | n.a. |
| 8 | | 10.963 | 596.425 | 2114.924 | 54.38 | 43.39 | n.a. |
| 9 | | 11.977 | 176.476 | 841.521 | 16.09 | 17.26 | n.a. |
| **Total:** | | | **1096.855** | **4874.292** | **100.00** | **100.00** | |

**Fig. 1. LC-MS measurement results of green tea extract sample**

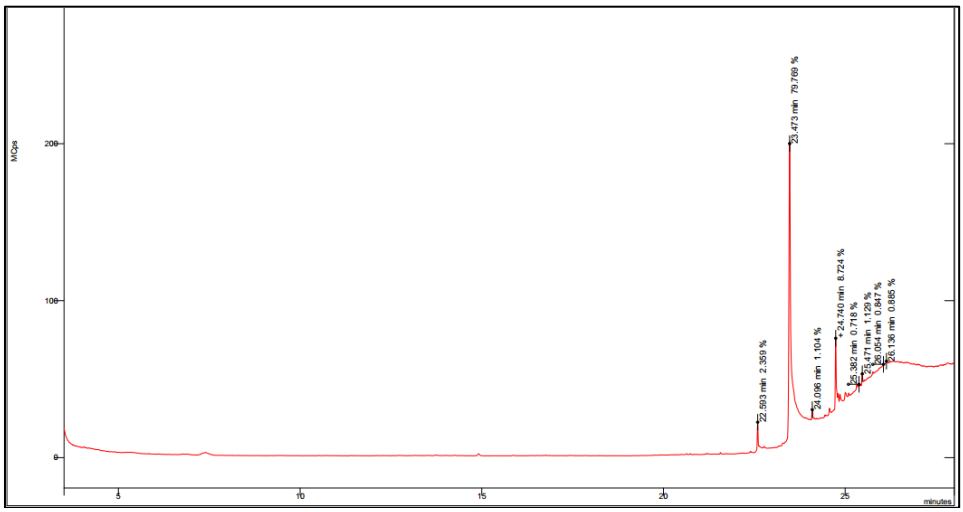



**Fig. 2. GC-MS result of *Oryza sativa* sample**

**Table 4. Chemical compositions of *Oryza sativa* sample**

| $N_o$ | RT | Identified compound | Percentage (%) |
|---|---|---|---|
| 1 | 22.593 | Palmitic acid | 2.41% ± 0.15 |
| 2 | 23.473 | Linoleic acid | 79.79% ± 0.22 |
| 3 | 24.096 | Glycidyl palmitate | 1.12% ± 0.12 |
| 4 | 24.569 | Undec-10-ynoic acid | 0.68% ± 0.11 |
| 5 | 24.740 | Methyl ester | 8.79% ± 0.09 |
| 6 | 24.808 | Glycidyl palmitoleate | 1.33% ± 0.23 |
| 7 | 24.859 | 2-Monopalmitin | 1.32 % ± 0.21 |
| 8 | 25.005 | Bis(2-ethylhexyl) phthalate | 1.12 % ± 0.25 |
| 9 | 25.471 | 2-Monoolein | 1.12% ± 0.12 |
| 10 | 26.136 | 24-Hydroxycalcidiol | 0.89% ± 0.18 |

*Note: The data are presented as mean ± standard deviation and P = .05*

**Table 5. Percentage of tyrosinase inhibition at the concentration of 100 ppm**

| Sample (*C. sinensis* : *O. sativa*) | Percentage (%) |
|---|---|
| 10:0 | 52.29% ± 0.33 |
| 8:2 | 46.58% ± 0.48 |
| 6:4 | 49.09% ± 0.15 |
| 5:5 | 56.76% ± 0.15 |
| 4:6 | 45.89% ± 0.32 |
| 2:8 | 41.11% ± 0.11 |
| 0:10 | 5.60% ± 0.11 |

*Note: The data are presented as mean ± standard deviation and P = .05*

**Table 6. Tyrosinase inhibitory activity of serum**

| Index | Water-based Serum | Oil-based Serum |
|---|---|---|
| Tyrosinase inhibition (%) | 18.7% ± 0.38 | 22.5% ± 0.41 |

*Note: The data are presented as mean ± standard deviation and P = .05*

At a concentration of 100 ppm, the percentage of tyrosinase inhibitory activity increased and peaked at a 5:5 ratio, demonstrating that there were interactions between green tea and rice bran that might influence the results. In addition, it has been reported that green tea extract has the ability in protection against the lipid oxidation of edible oils. The catechins in green tea extract might protect against lipid oxidation by donating electrons that stop the oxidative free radical chain reaction. As a result, green tea extract might inhibit the formation of rancid flavors, reduce the increase in peroxide value, and prevent any loss in fatty acids during the storage time, especially since the antioxidant activity of fatty acids was significantly protected by the presence of green tea extract in rice bran oil [18,19].

In this research, two serum products were formulated with the addition of the combined *C. sinensis* and *O. sativa extracts* (ratio of 5:5). It has been observed that the water-based serum was a homogeneous mixture having a consistent form of gel but remaining a liquid form. Therefore, it had a brown-green color. Meanwhile, the oil-based serum was a uniform combination with a gel-like consistency that was still liquid-like in nature. Due to the addition of cetyl alcohol to the oil-based formula, the texture was significantly thicker, and the color was slightly opaquer than the water-based product. It had a light green color. The water-based serums could be used throughout the day since it moisturizes and brightens the skin. It is suitable for persons with oily skin who want to prevent greasiness. Oil-based serum, on the other hand, contains essential fatty acids which nourish and moisturize skin. Therefore, the oil-based serum produced a finer texture after use than the water-based formula. In addition, the product formed a thin moisturizing film on the skin, making the skin feel stickier than when using water-based products. In addition, since jojoba and coconut oil was used as the base in oil-based serums, the oil-based serum might cover the skin's barrier against environmental pollutants, UV damage, and dry surroundings. This product might be used throughout the day or even at night, depending on when the skin needs more hydration. This oil-based serum might be ideal for persons with dry skin since the oil combination increases the skin's moisture content. Overall, the capacity of oil-based serum to inhibit tyrosinase was greater than that of water-based serum (Table 6). This is because coconut oil, a component in oil-based serum, might contribute to tyrosinase inhibitory activity.

## 4. CONCLUSION

The combination of *C. sinensis* and *O. sativa* extract with the ratio of 5:5 (w_w) had the highest percentage of tyrosinase inhibitory activity. In addition, water- and oil-based serum were successfully formulated containing 1% of combined *C. sinensis* and *O. sativa* extract with the ratio of 5:5 (w_w). These findings could be favorable for cosmetics development but need to be further investigated.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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