Characteristics and physiological effects of hydroponically cultured roots of the tea plant (*Camellia sinensis* L.)

ABSTRACT

Green tea has been reported to result in improvements in a range of health parameters. However, most research has only documented the effects of green tea brewed from leaves or leaf extracts. In addition to the leaves, the roots of tea plants also possess unique properties because of their requirements for growth, which may enable them to have useful physiological effects. We used a hydroponic system to grow the plants and explored the physiological effects of the roots, which biosynthesize one of the rarest functional amino acids, theanine (γ-ethylamide-L-glutamic acid). The level of theanine was much higher in the roots than in the leaves, and the roots also differed in other aspects of their chemical composition. We evaluated the effects of tea-root extract on the cognitive function and emotions of aged rats. Our results show that, in the object recognition test, aged rats drinking tea-root extract tended to show improved cognitive function and were more relaxed than the control group, which drank tap water. Furthermore, using a mouse model of human aging, we found that the average life span of mice that consumed the root extract was significantly increased. We suggest that tea roots contain unique components that may improve impaired physiological functions, and we therefore propose tea-root extract as a novel nutraceutical.

1. INTRODUCTION

Tea is one of the most widely consumed beverages in the world and has received attention because of its beneficial health effects, which include antioxidant, antimutation, anticancer, antihypertensive, anti-arteriosclerotic, anti-inflammatory, anti-obesity, antimetabolic syndrome, antibacterial, and antiviral activities [1]. Green tea, black tea, and oolong tea are all derived from the leaves of *Camellia sinensis,* but green tea has been the most studied for its physiological effects. Catechin, caffeine, and theanine (γ-ethylamide-L-glutamic acid) are the major components of green tea, providing astringency, bitterness, and umami, respectively. Recently, theanine has received attention because of its beneficial effects on the brain [2-5]. It is readily absorbed and permeates the blood–brain barrier, and it thus exerts effects in the brain, leading to reduced mental and physical stress, improved cognition, and improved mood [6,7]. Thus, tea leaves containing theanine have begun to receive attention for their potential use in our aging and stressed society. Theanine is more abundant in roots than in leaves or stems. It is biosynthesized from glutamic acid and ethylamine by γ-L-glutamylethylamide ligase in the roots and is then transported and stored in the leaves and stems. However, the

theanine present in leaves is decomposed and resynthesized to catechin with sun exposure [8]. Therefore, although technology has recently been developed for the industrial mass-synthesizing of theanine using microorganisms [9-11], the root of *Camellia sinensis* may serve as an alternative and abundant natural reservoir of theanine.

Theanine is one of the rarest amino acids and has been found in no other plants, except for some species belonging to the genus *Schima*, and only in one inedible mushroom [12]. Most research has reported the effects of theanine brewed solely from tea leaves or leaf extracts. Thus, we focused on tea roots and developed a hydroponic system to grow the plants and explore the physiological effects of the root extract. We assessed the properties of hydroponically cultured roots and their effects on cognition, emotion, and longevity.

2. PROPERTY OF TEA ROOTS OF HYDROPONICALLY GROWN TEA PLANT

2.1 Growth characteristics of tea plants

Hydroponic cultivation of tea plants was proposed by Konishi et al. in the 1980s as an effective method of cultivating plants and investigating their physiological effects [13]. Aluminum is toxic for most plants; however, it is an important growth element for tea plants [14]. Most plants release organic acids from the root that react with aluminum, which is a trace element in soil, and prevent its uptake [15-17]. We analyzed the release of major organic acids (malic, tartaric, oxalic, and citric acid) from the roots of hydroponically grown tea plants, but did not detect them in the solution [18]. However, it has been reported that *Camellia sinensis* absorbs aluminum from its roots and combines it with internal organic acids to inhibit the toxic effects of the aluminum [19-21]. Additionally, tea roots can directly absorb ammoniacal nitrogen, while other plants must enzymatically reduce it to nitrate nitrogen and then absorb and use it as a source of nitrogen for growth [18,22-24]. This mechanism may be used for the rapid biosynthesis of rare amino acids such as theanine [13,14,24]. Therefore, tea roots have specific features that differ from other plants, which are important not only for nutrient absorption but also for the physiological effects of tea on mammals.

2.2 Theanine production in tea roots

We used tea plants grown hydroponically for six months in a controlled environment for this study. The yield of roots produced depended entirely upon the growth of the plants [18,25].

The white rootlets of hydroponically grown plants are ideally suited to the analysis and biosynthesis of theanine: they contain about 10 g theanine per 100 g dry weight, a value three times higher than that for the lignified taproots, as shown in Table 1 [25]. By comparison, the typical theanine content of dried powdered leaves from plants cultivated in soil is about 1–2 g per 100 g. These results, which were obtained using liquid chromatography–mass spectrometry (LC/MS), indicate that the roots retain a large amount of theanine.

Table1 Concentration of theanine produced by different cultivation system

Sample	Cultivation	Conc. (g/100g)[a]
Leaves	Soil	1.30 ± 0.61[b]
Leaves	Hydroponics	1.45 ± 0.26
Lignified taproots	Hydroponics	3.33 ± 1.15
Fine white roots	Hydroponics	9.8 ± 1.75

[a] Values represent the means ± SEM (n=3).
[b] Max amount when plants were shaded and cultivated.

2.3 Chemical constituents of tea roots

We analyzed the amino acid content of the roots using an automatic amino acid analyzer, and we analyzed other components of the root extract using tandem LC/MS or inductively coupled plasma mass spectrometry. The composition

of major amino acids in the roots differed from that of standard tea leaves (Fig. 1A) [1]. The quantities of glutamic acid, aspartic acid, and serine were lower in roots than in leaves. In addition, the concentration of essential macrominerals differed in roots, where phosphoric acid content was high and potassium content was low (Fig. 1B). The ratios of essential trace minerals in the roots were also different from those in the leaves, although the overall composition was the same (Fig. 1C). One of the major differences between the roots and leaves is that the roots do not contain catechin or caffeine, which are beneficial ingredients of tea in terms of both flavor and its physiological effects. Tea roots may therefore have different physiological effects than leaves because of their unique chemical properties.
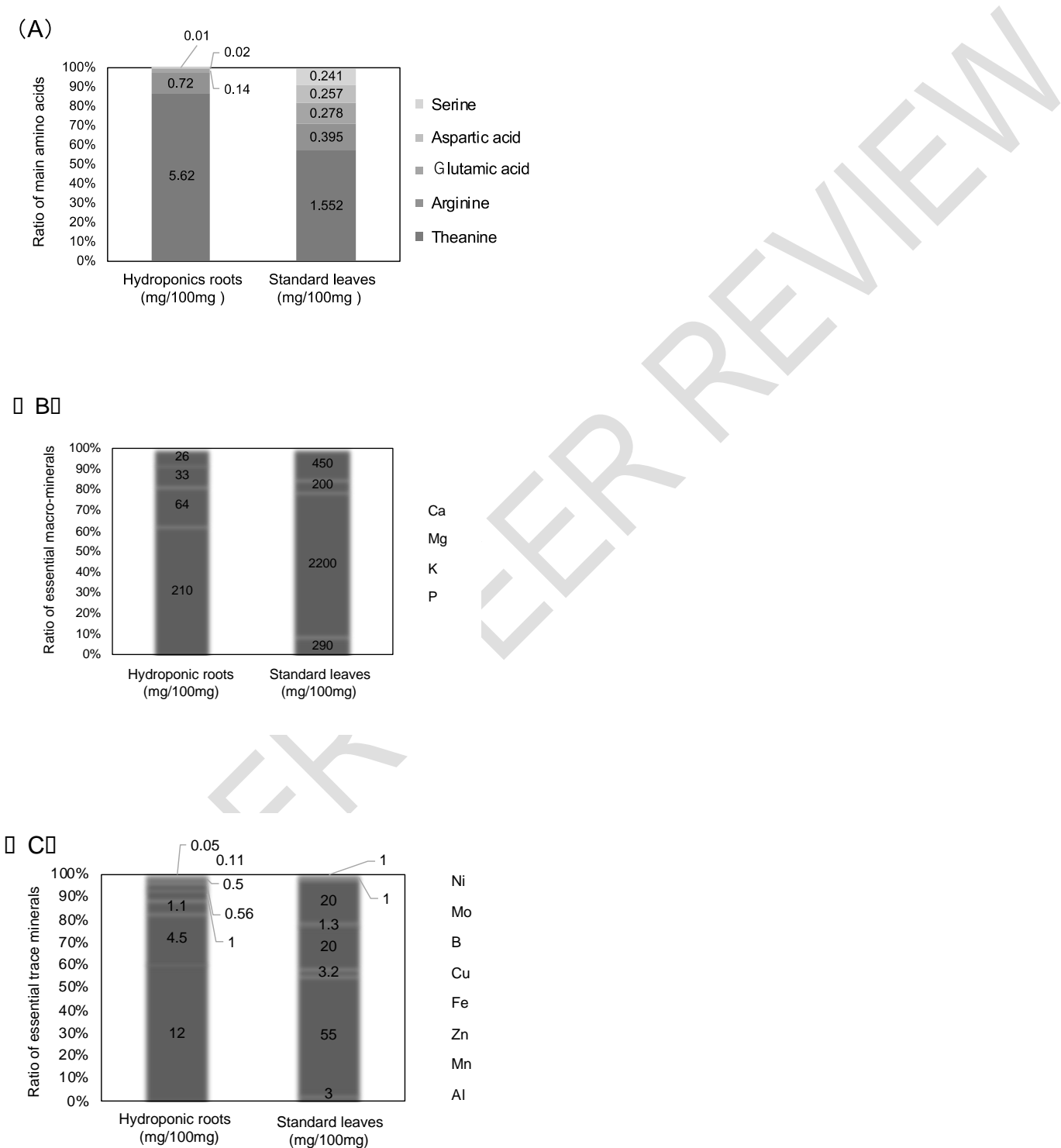
（A）

Ratio of main amino acids

Hydroponics roots (mg/100mg): Theanine 5.62, Arginine 0.72, Glutamic acid 0.14, Aspartic acid 0.02, Serine 0.01

Standard leaves (mg/100mg): Theanine 1.552, Arginine 0.395, Glutamic acid 0.278, Aspartic acid 0.257, Serine 0.241

Legend: Serine, Aspartic acid, Glutamic acid, Arginine, Theanine

（B）

Ratio of essential macro-minerals

Hydroponic roots (mg/100mg): P 210, K 64, Mg 33, Ca 26

Standard leaves (mg/100mg): P 290, K 2200, Mg 200, Ca 450

Legend: Ca, Mg, K, P

（C）

Ratio of essential trace minerals

Hydroponic roots (mg/100mg): Al 12, Mn 4.5, Zn 1.1, Fe 1, Cu 0.56, B 0.5, Mo 0.11, Ni 0.05

Standard leaves (mg/100mg): Al 3, Mn 55, Zn 3.2, Fe 20, Cu 1.3, B 20, Mo 1, Ni 1

Legend: Ni, Mo, B, Cu, Fe, Zn, Mn, Al

Fig. 1. Ingredient composition in tea roots cultivated hydroponically
  （A） Main amino acids
  (B) Essential macro-minerals
  (C) Essential trace minerals

3. PHYSIOLOGICAL EFFECTS OF TEA ROOTS IN AGED ANIMALS

3.1 Effect on recognition memory

To investigate the effect of tea root extract on the recognition memory of aged rats, we conducted an object recognition memory test (ORT), which is a task procedure that consists of three phases: habituation, acquisition, and test trials based on the animals' interest in novel and rare objects [26-28]. In this test, the rat is first introduced to two different objects. Then, after a delay, one of the two objects is replaced with a novel object and the rat is again exposed to them. If the animal remembers the previous exposure and recognizes which of the two objects is new, it will spend more time exploring that one. Longer exploratory behavior for a novel object than for an existing object indicates better cognitive ability. Thus, the effect of root extract on recognition memory can be assessed by measuring the duration of exploratory behavior. We divided the rats (males, Wister, 18–19 months old) into three groups (eight animals in each group), which were given the following treatments as their drinking water, which was supplied *ad libitum*: A, high-concentration tea-root extract (5 μg/mL theanine); B, low-concentration tea-root extract (0.5 μg/mL theanine); and C, tap water (control). The ORT was performed after the rats had been given these treatments for nine weeks. During habituation, the rats were allowed to freely explore an empty open-field box for 1 h on the day before the acquisition trial. During the acquisition trial, each rat was placed in the box with two different objects and allowed to explore freely for 5 min. Twenty minutes later, for the test trial, one of the objects in the box was replaced with a novel object and the rat was allowed to explore freely for 3 min. We filmed the rats' exploratory behavior during the acquisition and test trials and later analyzed the footage. Our results showed that during the acquisition trial, the rats in groups A and B traveled longer distances, spent more time away from the edges of the box, and exhibited higher activity levels than the control rats. Because rats exhibit anxiety-induced freezing when encountering novel situations, which reduces their activity level, our results suggested that the animals' high levels of activity in the box were due to the relaxing and anxiety-relieving effects of the root extract [29,30]. During the acquisition trial, the rats in groups A and B were in contact with the novel object for longer than the controls, showing that they recognized which of the two objects they had never seen before. Furthermore, the effect likely depended on the concentration of the root extract. There was no statistically significant difference in their performance, but drinking the extract did lead to some improvement in ORT results in aged rats.

3.2 Effect on life span

The effect of tea-root extract on longevity was analyzed using a mouse model of human aging (senescence accelerated mouse, SAM P10, male). The 10-week-old mice were divided into four groups, which were given the following treatments as their drinking water, which was supplied *ad libitum* until the end of the experiment (euthanasia): A, high-concentration tea-root extract (5 μg/mL theanine); B, low-concentration tea-root extract (0.5 μg/mL theanine); C, tap water (control); D, theanine (20 μg/mL theanine). The average lifespan of group A was significantly longer than that of the other groups (Fig.2). In addition, groups A and D had significantly longer lifespans than groups B and C. Previous studies have indicated that an intake of 20 μg/mL of theanine is effective for increasing lifespan and improving cognitive dysfunction [31-32]. The concentrations of theanine in the tea-root extract treatments we used were much lower than 20 μg/mL, yet the lifespan of the mice in group A was significantly extended relative to those in group D, which received a much higher concentration of theanine. Therefore, the positive effects of tea-root extract may be caused by the interaction of theanine with its other components. This may be due to differences in the ratio of other components of the roots relative to the leaves. Although the roots do not contain catechin and caffeine, which are well known to exert physiological effects both individually and together [33], the roots may contain previously unknown ingredients that have other positive effects on aged animals with impaired function.
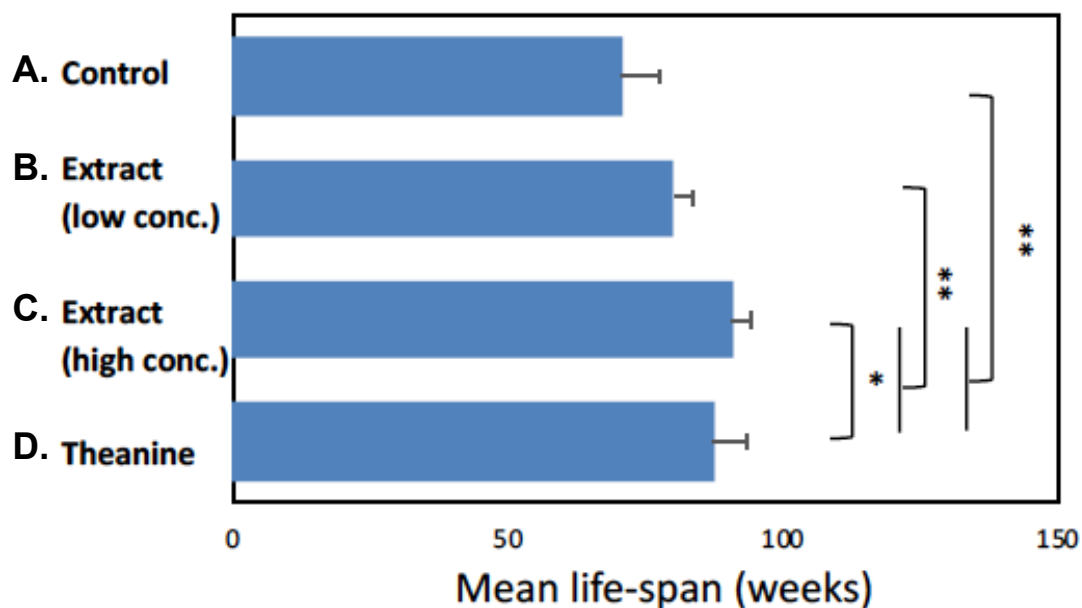
Fig 2: Mean life-span (Weeks)

4. MEDICINAL PERSPECTIVE ON TEA ROOTS

The brain consumes a large amount of oxygen, which results in the production of reactive active oxygen species (ROS) during metabolic processes. In addition, brain tissue is vulnerable to oxidative damage because it contains fewer antioxidant enzymes than other tissues [34-38]. SAM P10 mice produce more ROS in the brain than normal mice do, starting from an early age [39], and the activity of the antioxidant enzyme glutathione peroxidase is reduced in SAM P10 mice [40]. DNA oxidative damage is more likely to accumulate in these mice than in mice of other strains. Because theanine has no direct antioxidant effect, it may act indirectly to maintain the balance of ROS production and elimination in the brain, resulting in reduced oxidative damage [32]. Previous studies of the indirect effects of theanine on the brain support our ORT observations, which provide a measure of hippocampus-independent memory [32].

In these animal experiments, there was no significant difference in the intake of drinking water or in weight between groups, indicating that the root extract did not affect the eating or drinking habits of the animals. This indicates that the physiological effects observed were due to the root extract itself and that the extract did not negatively affect the health of the animals.

5. CONCLUSION

We have demonstrated novel properties of tea roots and their physiological effects on animal models. The effects of hydroponically grown roots have not previously been reported. Our findings suggest that hydroponic culturing could be a useful method for producing large quantities of naturally obtained theanine, as an alternative to industrially mass-manufactured theanine from microorganisms. Tea roots may offer other health benefits, resulting in a new type of pharmaceutical drug based not only on the function of theanine but also on possible synergies with other tea root components, ultimately improving both aging-related and other physiological conditions.

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