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Chemical analysis of polyphenols in camellia sinensis, their health benefits, and possibility for application in eye diseases

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Abstract: This article presents the results of a study on the determination of polyphenols in Black tea by high-performance liquid chromatography (HPLC). Polyphenols are bioactive compounds in plants, whose antioxidant properties have a therapeutic effect against various diseases. During the study, the main polyphenols (e.g. flavonoids and phenolic acids) in Black tea were identified and their quantitative indicators were determined. The effective effect of these compounds on eye diseases, in particular, age-related macular degeneration, glaucoma, and dry eye syndrome, was studied. The results showed that polyphenols in Karachay play an important role in protecting eye tissues from oxidative stress and reducing inflammatory processes. The results of this study serve to increase the pharmacological value of Karachay and expand the possibilities of its application in the healthcare sector.

Keywords: Polyphenol, phytochemicals, Camellia sinensis, Karachoy.

Introduction: Black tea (Camellia sinensis) contains many biologically active compounds, in particular

polyphenols, which are of great importance for human health. Polyphenols have antioxidant, anti-inflammatory, and cardioprotective properties and play an important role in the prevention and treatment of various diseases [1]. This study aimed to determine the amount of polyphenols in black tea and their health benefits using high-performance liquid chromatography (HPLC).

Black tea is translated from various languages as red tea - black tea, which has a more diverse variety and a stronger taste than other teas. The name of the drink, which first appeared in China, is hong cha ("red tea") due to the color of the leaves that oxidize when properly processed. Today, it is widely consumed and harvested, including in China, Japan, Korea, and Singapore. While green tea usually loses its flavor within a year, black tea retains its flavor for several years [2].

A study by Chinese scientists at National Chiayi University, led by Chih-Yu Lo and Min-Hsiung Pan, investigated the nutritional value of tea, mainly its polyphenols and antioxidant properties. A meta-analysis of observational studies concluded that black tea consumption does not affect the development of oral cancer, Asian esophageal or prostate cancer, or lung cancer in Asian or Caucasian populations. In addition, black tea contains polyphenols - antioxidants that help maintain the balance of intestinal microflora and eliminate pathogenic bacteria in the body [3].

Cleans the mouth According to scientific research, the polyphenols in black tea protect against oral diseases, rashes, and stomatitis. Benefits for the heart European scientists have proven that those who drink 3-4 cups of tea a day have a 21% lower risk of heart disease. True, it is not recommended to drink black tea in a bitter form. But if it is consumed in moderation, there is no need to worry [4]. Cancer prevention According to reports, the polyphenols and catechin antioxidants in black tea act as a preventive measure for certain types of cancer. A scientific study conducted by European scientists showed that women who drink black tea have a lower risk of developing uterine cancer than others. Strengthens bones The phytochemicals in this drink protect against bone fragility. Therefore, arthritis is less common among black tea lovers. Improves immunity Alkyloamine antibodies stimulate the immune system, while antioxidants provide resistance to viruses. So, hot black tea is also good for colds, flu, or coughs [5].

Black tea is widely used as a natural remedy for eye diseases. The tannins and antioxidants in black tea have antimicrobial effects. This is especially useful for mild eye infections (conjunctivitis, etc.). Applying cold black tea bags to the eyes reduces swelling and redness. This is especially useful for tired or sleep-deprived eyes. A cooled infusion of black tea reduces redness in the eyes and soothes the eyes [6]. It can also be used in case of allergies or irritation. Black tea bags or compresses stimulate blood circulation, which has a positive effect on the skin around the eyes. The light hydrating properties of black tea help moisturize the eyes. Washing or compressing with tea leaves reduces eye dryness.

Experience part

Reagents and equipment used. Gallic acid was obtained from Macklin (China), Salicylic acid from Rhydburg Pharmaceuticals (Germany), quercetin, apigenin, and kaempferols from Regal (China), and rutin were isolated from natural sources by extraction and column chromatography. Water, acetonitrile, acetic acid of the chemically pure brand, and sodium hydroxide were used as reagents of HPLC purity.

The content of polyphenols in the plant was determined using an LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu, Japan.

Preparation of standard solutions. Gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), kaempferol (5 mg) were dissolved in 96% ethanol for 20 minutes in an ultrasonic bath and transferred to a 50 ml flask and made up to the mark with ethanol. 200 µl of each solution was taken, mixed, and diluted to prepare a total of 4 different solutions. Each solution was poured into a vial and used for analysis.

Preparation of plant extract. For the extraction of phenolic compounds, 1 g of the test sample was weighed with an accuracy of 0.01 g on an NV222 balance manufactured by OHAUS (USA), placed in a 50 ml conical flask, and 25 ml of 96% ethanol was added. The mixture was extracted in an ultrasonic bath of the GT SONIC-D3 brand (China) at a temperature of 60 C for 20 minutes. Then the mixture was cooled, filtered, and made up to 25 ml with ethanol in a volumetric flask. 1.5 ml of the extract was centrifuged at 7000 rpm in a Mini-7 brand (BIOBASE, China) centrifuge and filtered through a 0.45 µm syringe filter, and used for analysis.

Chromatographic conditions

Determination of phenolic compounds. Standard solution, sample extract Shim pack GIST C18 reversed-phase column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) and a gradient mobile phase consisting of acetonitrile (A) and 0.5% acetic acid in water (B) (Table 1) were used. The injection volume was 10 µl, the flow rate was 0.5 ml/min, and the column thermostat was set to 40 °C. The analytical signal (peak area) of phenolic compounds

was recorded at 300 nm (Figure 1).

Table 1. Mobile phase gradient program.

Time	Acetonitrile (A), %	0,5% acetic acid (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	Termination	

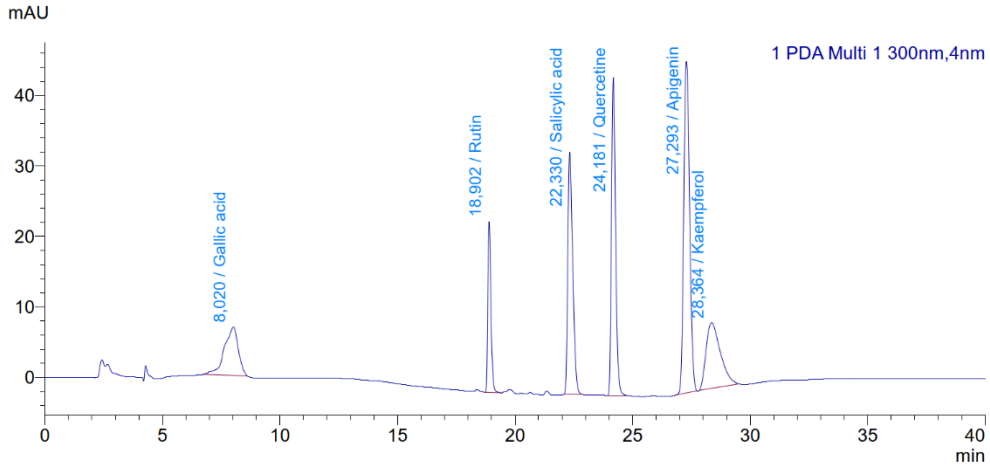

Figure 1. Chromatogram of standards at 300 nm.

RESULTS

Determination of the amount of phenolic compounds in the sample extract of Black tea. A chromatogram of a sample extract weighing 1 g was obtained (Figure 2), and based on the results, the amount of phenolic compounds in 100 g of the sample was calculated using the following formula and presented in Table 3.

$$X = \frac{C_{phen} \cdot V_{ekstrakt}}{m_{example}} \cdot 100\ g$$

Here, X – the amount of phenolic compounds in 100 grams of plant material, mg;

C_{phen} – the concentration of phenolic compounds in the extract determined by the HPLCH method, mg/l;

$V_{extract}$ – the volume of the sample extract, l;

example – the mass of the sample taken for extract preparation.

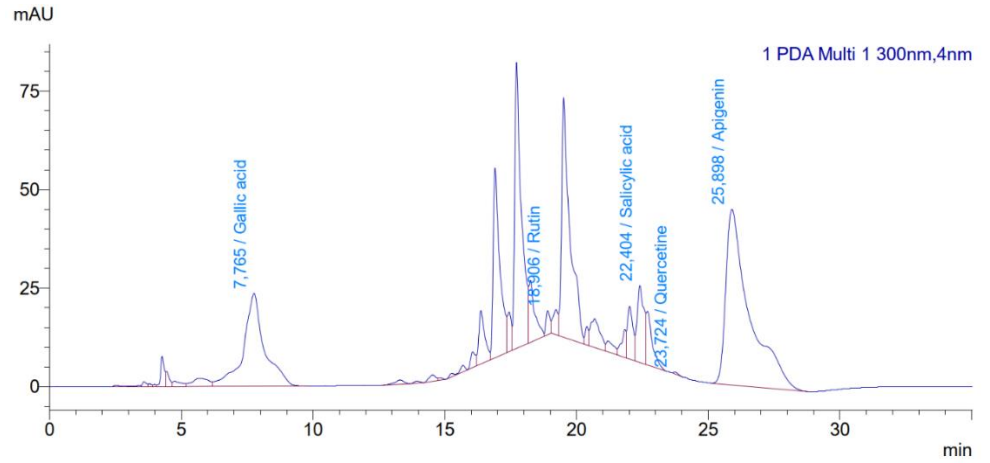

Figure 2. Chromatogram of polyphenols in the sample extract.

Table 2. Amount of polyphenols in the extract and retention times.

Phenol compound name	Catch time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Gallic Acid	7,765	61,433	153,583
Rutin	18,906	4,935	12,338
Salicylic Acid	22,404	12,472	31,180
Quercetin	23,724	0,175	0,438
Apigenin	25,898	58,004	145,010
Kaempferol	Not specified	0	0,000

From Table 1 above, it can be seen that the content of gallic acid (153.583 mg/100g) in the “Black tea” plant is significantly higher than that of others. It is also known that the content of apigenin, rutin, and salicylic acid in “Black tea” is also significantly higher.

CONCLUSION

Determining the content of polyphenols in Karachoy using the HPLC method is a reliable tool for studying the biological significance of these substances. The results obtained show that polyphenols in Karachoy can play an important role in supporting human health, in particular, in the prevention and treatment of eye diseases. The beneficial effects of gallic acid and apigenin in eye diseases are manifested through their biologically active properties.

Gallic acid reduces oxidative stress by neutralizing free radicals in the eye. Oxidative stress is a major factor in eye diseases associated with clouding of the vision, such as macular degeneration, cataracts, and diabetic retinopathy. Gallic acid has the ability to reduce inflammation, which is beneficial in uveitis, dry eye syndrome, and other inflammatory diseases. Its ability to repair blood vessels and improve microcirculation helps improve blood circulation in diabetic retinopathy and glaucoma. Apegnin has a healing effect on uveitis, conjunctivitis, and dry eye syndrome by reducing inflammation. This substance is effective in blocking the activity of inflammatory agents. Apegnin helps to prevent cataracts and macular degeneration by neutralizing free radicals, and apegenin helps to preserve vision in glaucoma by reducing intraocular pressure and improving blood circulation.

Apigenin and gallic acid, when used in combination, effectively reduce oxidative stress and inflammation in eye tissues. They can be used to: slow the development of cataracts, prevent macular degeneration and glaucoma, improve visual function, and eliminate dry eye syndrome and inflammatory diseases. Timely and targeted use of these substances is important in the prevention and therapy of eye diseases. The combination of these two substances can be effective in treating and preventing many health problems for humans. Since the consumption of Black tea is significant in supporting a healthy lifestyle, it is recommended to include it in the regular diet. In the future, it is advisable to study polyphenols in more depth using other biochemical methods.

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