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Full Length Research Paper

Comparison of antibacterial activity of water and ethanol extracts of *Camellia sinensis (L.)* Kuntze against dental caries and detection of antibacterial components

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Present study describes the antibacterial activity of green tea extracts against dental caries. Green tea samples were collected from local market of Lahore. The antibacterial activity of water and ethanol extract of *Camellia sinensis (L.)* Kuntze were examined against *Lactobacillus acidophilus and Streptococcus mutans*. Antibacterial activity *was* investigated by paper diffusion test and Minimum inhibitory concentration. Water extract of *C. sinensis* produce zones of inhibition 6 to 18 mm and 8 to 27 mm, respectively against *L. acidophilus* and *S. mutans.* Ethanol extracts of C*. sinensis* produced larger zones of inhibition 15 to 33 mm and 19 to 35 mm, respectively against *L. acidophilus* and S*. mutans*. The minimum inhibitory concentration (MIC) for the green tea water extract against *L. acidophilus* and *S. mutans* extract was 0.9 and 0.8 mg/ml, respectively. Minimum inhibitory concentration (MIC) for the green tea ethanol extract against *L. acidophilus* and *S. mutans* extract was 0.7 and 0.7 mg/ml, respectively. Results showed that ethanol extracts of green tea exhibited greater antibacterial activities against *L. acidophilus* and *S. mutans* than water extracts. Active compounds in green tea extract were determined by high pressure liquid chromatography. Metals analyzed by Atomic abortion spectrophotometer were Ag, Pb, Na and Cr. The solubility of studied metals in tea water extracts varied widely, ranging from 0.00 to 1.477 mg/L. The concentration of metals Ag, Na, Cr and Pb were detected in the following order 1.477>0.100>0.0096>0.00 mg/ml. Fluoride ion in green tea was 2.8 and 2.1 ppm for water and ethanol extract of *C. sinensis*, respectively.

Key words: *Camellia sinensis, Lactobacillus acidophilus, Streptococcus mutans*.

INTRODUCTION

Natural products have been used for thousands of years in folk medicine for several purposes. As most of the oral diseases are due to bacterial infections and it has been well documented that medicinal plants confer considerable antibacterial activity against various microorganisms including bacteria responsible for dental caries (Anna et al., 2000). Dental caries is one of the most common infectious diseases of man, particularly in deprived urban industrial areas. There is convincing evidence that the bioactive components of green tea are able to influence the process of caries formation at several different stages: they may inhibit proliferation of the streptococcal agent, interfere with the process of adhesion to tooth enamel or act as inhibitors of glucosyl transferase and amylase (Peter et al., 2005).

Effective antimicrobial agents against these oral pathogens could play an important part in the prevention of dental caries. However, many attempts for prevention of dental caries were of no practical use up to the present. Antibiotics such as penicillin and erythromycin have been reported to effectively prevent dental caries in

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animal and humans but they are never used clinically because of many adverse effects such as hypersensitivity reaction, suprainfections. Tea polyphenols which are constituents of tea extracts have previously been shown to have antibacterial activities against human and animal disease-related bacteria, phytopathogenic bacteria and food-borne bacteria (Aizawa et al., 1996). The leaves (*Camellia sinensis*) are source of such mineral elements as essential for health: Zinc, Manganese, Iron, Magnesium, Copper, Titanium, Aluminum, Bromium, Sodium, Potassium as well as nickel, chromium and also phosphorus (Fernandez et al., 2001; Grag et al., 2005; Gramza et al., 2005; Chu et al., 1997; Ferrara et al., 2001; Kok and Tasciogl, 1998; Hui, 1992). The antibacterial activity of silver has long been known and has found a variety of applications because its toxicity to human cells is considerably lower than to bacteria. Silver itself is not toxic (Khan et al., 2008). Bacterial inhibition depends upon the concentration of silver nanoparticles and the number of bacterial cells. It reflects that silver nanoparticles have a significant biocide effect in reducing bacterial growth for practical applications.

The objectives of this study were to assess the antibacterial activity of the green tea ethanol and water extract against the oral bacteria *Streptococcus mutants*, and *Lactobacillus acidophilus.* To determine the antibacterial component responsible for antibiotic activity.

MATERIALS AND METHODS

Plant collection

Lipton brand green tea which is commonly consumed in Pakistan was purchased from local market of Lahore.

Water extraction

Green tea (5, 10, 15 and 20 g) were boiled in 150 ml of distilled water for 15 min and filtered. Green tea extract with different concentration was made by keeping the volume of distilled water constant.

Ethanol extraction

In Ethanol extraction of green tea by soxhlet as described by (Moon et al., 2005) in which 20 g of green tea leaves were soxhlet extracted using 1000 ml ethanol. The extraction lasted for two hours and ethanol was evaporated on rotary evaporator. The powdered ethanol extract was kept frozen at 15°C until further use. Rotary evaporator (EYELA model no A-1000S, Japan) was used to separate green tea extract into its components based on their respective volatilities, through the process of evaporation and condensation. The ethanol extraction of the active ingredient of the leaves was carried out at 65°C.

Organism

The strains used in this work were *L. acidophilus* and *S. mutans. L. acidophilus* was isolated from yogurt and *S. mutans* were isolated from dental caries.

Collection of bacterial sample

Lactobacillus was isolated from yogurt after giving heat shock at 80°C for 15 min and then rapidly cooled to 0°C in an ice bath. The nutrient agar was used for isolation of bacteria by pour plate method. The sample was streaked on nutrient agar plate and incubated at 37°C for 24 h. Blood agar was used to distinguish *S. mutan*s from dental caries. Collected bacteria were cultured in the nutrient broth at 37°C over night.

Preparation of inoculums

All the bacterial species were grown in nutrient broth in 250 ml cotton plugged conical flask. A loop full of pure bacterial culture was taken and suspended in 25 ml nutrient broth in 250 ml conical flask. The flask was incubated at 37°C.

Antibacterial susceptibility testing

The antibacterial tests of the leaf extracts were tested against *L. acidophilus and S. mutans* using paper disc diffusion inhibition test (Mbata et al., 2008). In the paper disc diffusion test, Whatman filter paper discs were soaked in the leaf extract for 30 min. 10.0 µL of a 24 h broth culture of the bacteria specie was spread on the surface of nutrient agar plates. The paper discs containing the extracts were placed at different areas on the surface of each plate. The plates were incubated at 37°C for 24 h. After 24 h antibacterial activity of the extract against the test bacteria was observed by growth free zone of inhibition near the respective disc.

Minimum inhibitory concentration

For measurement of the minimum inhibitory concentration of green tea extract according to the agar diffusion method. The extracts were incorporated into nutrient broth at concentration ranging from 0.1 to 1.0 mg/ml. A control tube containing the growth medium and the bacteria was set-up. The mixtures were incubated at appropriate temperature of 37°C for 24 h. Next day 10 micrometer from these test tubes were streaked on the nutrient agar Petri plates and incubated at 37°C for 24 h.

Metal analysis

Metal analysis method used for metal detection in green tea (Tojiro and Tadakazu, 2006). Atomic absorption spectrophotometer (Thermo electron corporation VD100, UK) was used for heavy metals detection (Ag, Pb, Na and Cr).

Fluoride ion analysis

Fluoride ion-selective electrode method (Samuel et al., 2010)

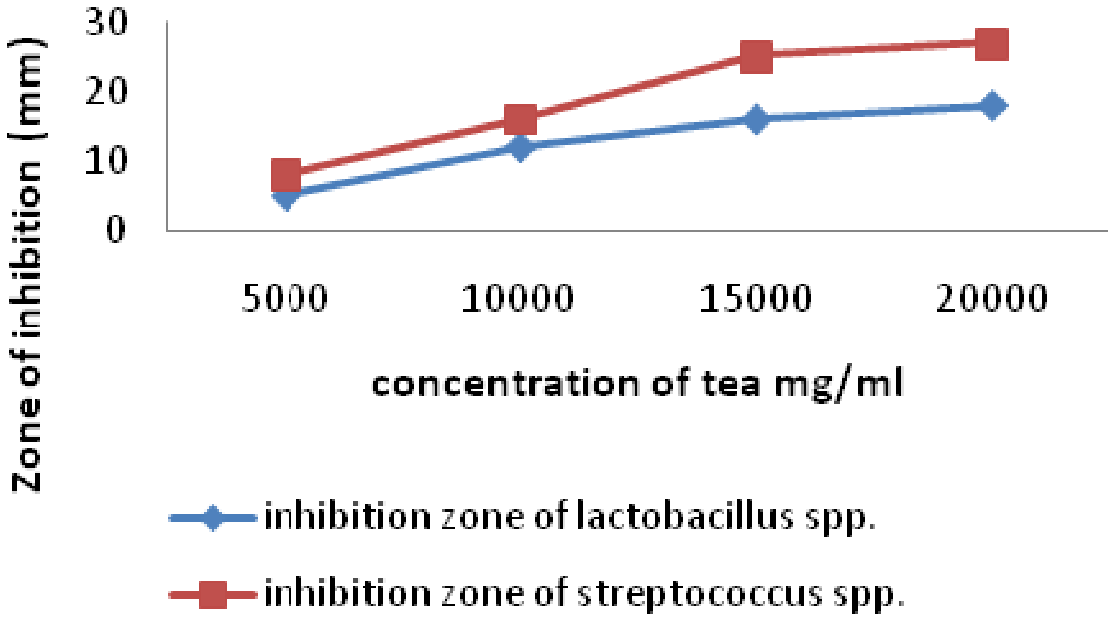

Figure 1. Antibiotic assay of *C. sinensis* (water extract) against *Lactobacillus spp.* and *streptococcus* spp.

(Meter lab ion450, France) was used for fluoride ion analysis in green tea extracts.

Identification of Favoinoids in the green tea infusion by HPLC-UV

Identification of Favoinoids in the green tea infusion has been performed by HPLC-UV (Moon et al., 2005). High pressure liquid chromatography (LC.20AT SHIMADZU Japan) equipped with UV detector. Mobile phase was Mathanol with buffer ammonium acetate pH 4.0 and acetonitrile with buffer CH_3COONH_4 were used as eluting solvent with a flow rate 0.5 ml/min and detection wavelength was 280 nm. The chromatic data was obtained by PC system.

RESULTS AND DISCUSSION

Antibiotic assay of *C. sinensis* (water extract)

Antibiotic activity of green tea (water) extract was studied against *L. acidophilus* and *S. mutans*. Concentration of green tea in water was varied from 5000 to 20000 mg.

Effects of concentration of green tea water extract against *Lactobacillus acidophilus* and *Streptococcus mutans*

The data of (Figure 1) shows inhibitory zones increased with the increasing concentration of green tea water extract. Inhibition zones of diameter 5, 12 and 16 mm were observed at 5000, 1000 and 15000 mg/ml green tea extract, respectively (Figure 1). Maximum zones of inhibition (18 mm) against *L. acidophilus* were obtained at 20000 mg/ml tea concentration.

Inhibitory zones increased with the increasing concentration of green tea water extract. Inhibition zones of diameter 8, 16 and 25 mm were observed at 5000, 1000 and 15000 mg/ml green tea extract, respectively. Maximum zones of inhibition (27 mm) against S. *mutans* were obtained at 20000 mg/ml tea concentration. Water extract of leaves of *C. sinensis* possess greater antibacterial properties against *S. mutans.*

Effects of concentration of green tea ethanol extract against *Lactobacillus acidophilus* and *Streptococcus mutans*

Antibiotic activity of green tea (ethanol) extract was studied against *L. acidophilus* and *S. mutans.* Concentration of green tea in water was varied from 5000 to 20000 mg.

The inhibitory zones increased with the increasing concentration of green tea ethanol extracts (Figure 2). Inhibition zones of diameter 15, 20 and 29 mm were observed at 5000, 10000 and 15000 mg/ml green tea (ethanol) extract, respectively (Figure 2). Maximum zone of inhibition (33 mm) against *L. acidophilus* was obtained at 20000 mg tea concentration. Ethanol extract of the leaves of *C. sinensis* possess greater antibacterial properties against *L. acidophilus.*

The inhibitory zones increased with the increasing concentration of green tea ethanol extracts. Inhibition zones of diameter 19, 24 and 34 mm were observed at 5000, 10000 and 15000 mg/m l green tea (ethanol)

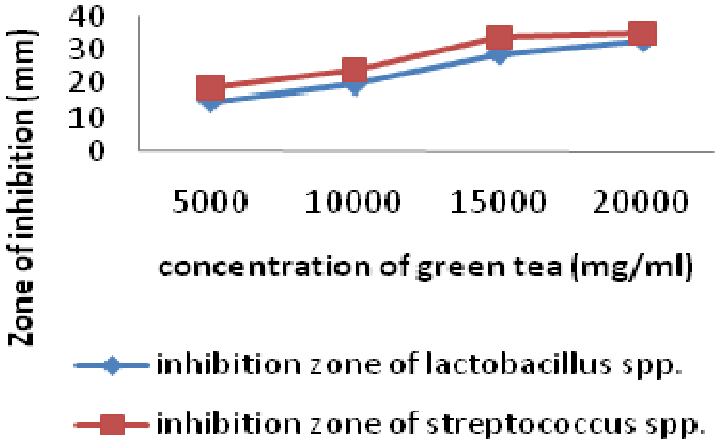

Figure 2. Antibiotic assay of *C. sinensis* (ethanol extract) against *Lactobacillus acidophilus* sp. and *Streptococcus mutans.*

Table 1. Minimum inhibitory concentration (MIC) of *C. sinensis* (water) extract against *lactobaciilus acidophilus Streptococcus mutans'* sp.

S/N	Minimum inhibitory concentration mg/ml	Colony count CFU/ml of *Lactobacillus* spp.	Colony count CFU/ml of *Streptococcus* spp.
1.	0.1	139	140
2.	0.2	100	110
3.	0.3	87	85
4.	0.4	70	74
5.	0.5	51	13
6.	0.6	10	4
7.	0.7	3	4
8.	0.8	3	3
9.	0.9	2	3

extract, respectively. Maximum zone of inhibition (35 mm) against *S. mutans* was obtained at 20000 mg/ml tea concentration. Ethanol extract of the leaves of *C. sinensis* possess greater antibacterial properties against *S. mutans.*

Minimum inhibitory concentration (MIC) of water extracts against *Lactobacillus acidophilus* and *Streptococcus mutans*

Minimum inhibitory concentration (MIC) of green tea (water) extract against *L. acidophilus and S. mutans* was determined. Green tea concentration varied from 0.1 to 0.9 mg/ml. Minimum inhibitory concentration of green tea (water) extract studied against *L. acidophilus* (Table 1). The 0.1 mg/ml of green tea extract did not demonstrate any significant antibacterial activity against *L. acidophilus.* Minimum inhibitory concentration of green tea (water) extract against *L. acidophilus* was 0.9 mg/ml.

Minimum inhibitory concentration (MIC) of green tea (water) extract studied against *S. mutans* (Table 1). Green tea concentration varied from 0.1 to 0.9 mg/ml. The 0.1 mg/ml of green tea extract did not demonstrate any significant antibacterial activity against *S. mutans.* Minimum inhibitory concentration of green tea (water) extract against *S. mutans* was 0.8 mg/ml. Extracts of green tea strongly inhibited *Escherichia coli, Streptococcus salivarius* and *Streptococcus mutans*, suggest that green tea is a potent inhibitor of oral streptococci and effective potential sources of natural antioxidants (Tzung et al., 2008; Azmat and Mujtaba, 1998).

Minimum inhibitory concentration (MIC) of Ethanol extracts against *Lactobacillus acidophilus and Streptococcus mutans*

Minimum inhibitory concentration (MIC) of green tea

Table 2. Minimum inhibitory concentration (MIC) of C. *sinensis* (ethanol) extract against *lactobaciilus acidophilus Streptococcus mutans' sp.*

S/N	Minimum inhibitory concentration mg/ml	Colony count CFU/ml of *Lactobacillus acidophilus*	Colony count CFU/ml of *Streptococcus mutants*
1.	0.1	141	134
2.	0.2	109	106
3.	0.3	100	83
4.	0.4	91	68
5.	0.5	72	56
6.	0.6	54	11
7.	0.7	5	3
8.	0.8	5	3
9.	0.9	5	3

(ethanol) extract against *L. acidophilus and S. mutans* was determined. Green tea concentration varied from 0.1 to 0.9 mg/ml. Minimum inhibitory concentration of green tea (ethanol) extract studied against *L. acidophilus* (Table 2). Green tea concentration varied from 0.1 to 0.9 mg/ml. The 0.1 mg/ml of green tea extract did not demonstrate any significant antibacterial activity against *L. acidophilus*. Minimum inhibitory concentration of green tea (water) extract against *L. acidophilus* was 0.7 mg/ml.

Minimum inhibitory concentration of green tea (ethanol) extract studied against *S. mutans* (Table 2). The 0.1 mg/ml of green tea extract did not demonstrate any significant antibacterial activity against *S. mutans.* Green tea concentration varied from 0.1 to 0.9 mg/ml. Minimum inhibitory concentration of green tea (water) extract against *S. mutans* was 0.7 mg/ml. Tea polyphenols and polyphenols constituents, especially epigallocatechin gallate (EGCG), were significantly higher in the ethanol extract of green tea than in the aqueous extract, which were possibly responsible for the higher antioxidant activity of the ethanolic extract (Feng et al., 2008).

Identification of Flavonoids in the green tea infusion by HPLC-UV

Catechins were detected, but it was overlapped with some impurity peaks and was not determined. The chromatogram of the green tea extract at 100°C for 15 min was comparatively showed (Figure 3). Active compounds of green tea extract were compared to the standard mixture chromatogram (Kim et al., 1989) to identify corresponding flavonoids. Active compounds were identified in the green tea extracts with UV detector.

Fluoride content in green tea extract

Substantial fluoride levels were found in all tea samples. The mean fluoride concentrations in tea were 2.1 for ethanol extract and 2.8 ppm for water extract, respectively showed (Table 3).

Metal content in green tea extract

The tea brands selected from Lahore markets contain considerable contents of the metals. The solubility of studied metals in tea water extracts varied widely and ranged from 0.0 to 1.477 mg/ml. The concentration of metals Ag, Na, Cr and Pb lies in the following order 1.477>0.100>0.0096>0.00 mg/ml (Table 4). The concentration of toxic metal Pb in tea is too low. On the other hand, the concentration of non toxic metals like Ag, Na and Cr lies within the acceptable daily intake. Present results revealed that Ag in tea samples is relatively higher than other heavy metals. Since ancient times, the silver ion has been known to be effective against a broad range of microorganisms.

Today, silver ions are used to control bacterial growth in a variety of medical applications, including dental work, catheters, and the healing of burn wounds (Klasen, 2000; Gin et al., 2005; Schreurs and Rosenberg, 1982). The antibacterial investigations were performed in solution and on Petri dishes. The silver nanoparticles were found to exhibit antibacterial effects at low concentrations (Baker et al., 2005). The possible daily allowances of silver was 0.035 mg for the man and 0.040 mg for the woman, the man excreted, on average, 50% more silver than he ingested (no explanation of this anomaly was given), whereas the woman retained an average of 16% of the silver ingested.

In the face of these conflicting data, the WHO has assumed that approximately 10% of silver ingested is absorbed. Green tea and their major constituents such as catechins are famous materials for their antioxidative and anti-carcinogenic activity, but many compounds with reducing power can promote the oxidation in their oxidized form or in the presence of metal ion (Kawan and Park, 2005).

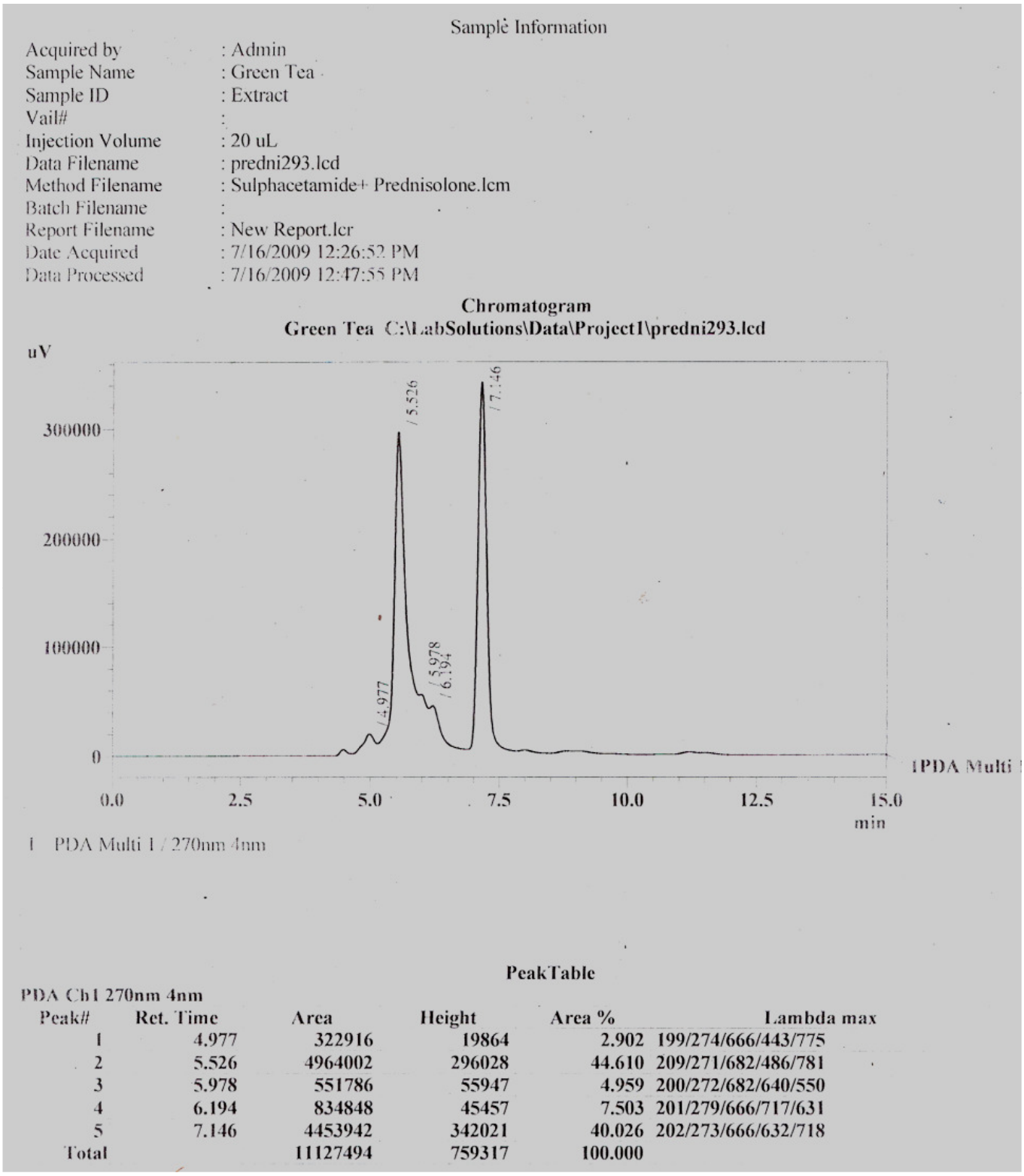

Figure 3. The chromatogram of the active compounds (270 ppm) in green tea extract (at 40°C for 15 min) by UV detection.

Conclusions

Ethanol extract of *Camellia sinensis* was found to be more effective then water extract. It can be used as mouth wash. The elemental analysis results showed that tea serves as one of the source of human intake of

Table 3. Fluoride content in *C. sinensis* extracts.

S/N	Tea samples	Fluoride content (ppm)
1.	Water extract	2.8
2.	Ethanol extract	2.1

Table 4. Metal contents mg/L in tea *C. sinensis* (water) extract.

S/N	Tea samples	Ag (mg/L)	Na (mg/L)	Cr (mg/L)	Pb (mg/L)
1.	GT1	1.4770	0.1000	0.0096	ND
2.	GT2	1.3924	0.1942	0.0065	ND
3.	GT3	1.2036	0.1367	0.0086	ND
4.	GT4	1.3006	0.7650	0.0008	ND
5.	GT5	1.1027	0.5622	0.00100	ND

ND= not detected, GT= green tea.

different metallic elements, which are essential for humans up to certain level. Results showed that fluoride in tea sample relatively lies within the acceptable daily intake which is also responsible for the prevention of dental caries.

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