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Antifungal activity of green tea leaves (*Camellia sinensis* L.) sampled in different harvest time

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ABSTRACT

Antifungal activity of green tea leaves (*Camellia sinensis* L.), sampled 3 different harvest time (May, July and September) from Rize region in Turkey, against a number of fungi (*Candida albicans, Candida glabrata, Candida kruseii, Candida parapsilosis* and *Candida dubliensis*) has been investigated. In addition, the catechin-based flavonoids in green tea leaves such as epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epicatechin (EC) were determined. All methanol extract of green tea samples showed a broad-spectrum antifungal activity against all *Candida* species in broth microdilution bioassays. However maximum activity of methanol extract (>17 mm inhibition zone) was observed against *Candida albicans* at 3rd harvest time. In general, antifungal activity increased from 1st harvest time to 3rd harvest time. Catechin-based flavonoids have also increased from 1st harvest to 3rd harvest indicating a correlation with antifungal activity.

Keywords: Antifungal activity, *Camellia sinensis*, flavonoids, seasonal variation.

INTRODUCTION

Tea (*Camellia sinensis* L.) is one of the most popular beverages in the world and it is originated in China, dates back several thousand years ago (1). Among tea producer countries, Turkey ranks 5th place with approximately 1.000.000 tons fresh leaves and 200.000 tons dry tea productions (2). Commercial tea production in Turkey just started around 1960s and it developed very quickly. Rize located in Northeast part of the country is accepted the capital of tea in Turkey. Tea is drunk in almost every part of Turkey and accepted one of the most important social and medicinal beverages. It is well known that, since 3000 B.C., traditional Chinese medicine has recommended green tea for headaches, body aches and pains, digestion, enhancement of immune system, detoxification, as an energizer and to prolong life (3). The health benefits of tea are confirmed and therapeutic value of tea for the prevention and treatment of many diseases has become more and more commonly known (4).

The wearing of prosthetic appliances is commonly associated with denture stomatitis, a bacterial and fungal originated disease, characterized by an inflamed mucosa, particularly under the upper denture (5). The fungi, *Candida albicans* is the most frequently isolated from the oral cavities of such kind of patients (6, 7, 8, 9). Denture stomatitis is a debilitating oral disease that can drastically reduce the quality of life for some individuals. Many denture wearers are also unaware that they have oral candidosis, and this could potentially lead to long-term complications if their immune status were to alter (10).

Widespread emergence of microbial (bacteria, fungi, virus etc.) resistance to present drugs represents a serious problem in treatment of such kind of infections (11). More recently an increased interest to use natural antimicrobial compounds, like plant extracts of medicinal plants possess a characteristic flavour and sometimes show antimicrobial activity (12). For centuries, medicinal plants have been used in traditional medicine for curing various diseases (13). Plants can produce many compounds and secondary metabolites which has antimicrobial properties (14).

Tea leaves contain more than 35% of their dry weight in polyphenols. Non-fermented green tea contains predominantly flavanols, flavandiols and phenolic acids like gallic acid, coumaric acid or caffeic acid, with those in green tea being higher than those in black tea (15). Flavonoids are natural polyphenolic substances are common constituents in tea leaves as catechin groups. Dietary intake of catechins displayed various benefical effects such as antimicrobial effects (16).

There were studies related to antimicrobial activity of tea leaves (16, 17). However the effect of different harvest time on antimicrobial activity against a number of microorganisms and seasonal variation of catechin groups in tea leaves in Turkey has not been studied so far.

MATERIALS AND METHODS

Collection and preparation of tea leaf samples

Green (Fresh) tea flush (two leaves and one bud) harvested from seed propagated tea plants (*Camellia sinensis*) from Ikizdere-Rize region at 3 main harvest period (May, July, September) in 2008 year. The green tea were extracted with methanol then the extracts filtered using Whatman filter paper (No:1) and stored in a freezer at − 80 °C until antifungal tests.

Fungal species

A total 5 *Candida* isolates (*Candida albicans*, *Candida glabrata, Candida kruseii, Candida parapsilosis* and *Candida dubliensis*) were used for antifungal tests. The pathogenic fungi were obtained from the culture collection at Ataturk University (Faculty of Medicine). Cultures of each of the fungi were maintained on Subouraud-Dextrose- Agar (SDA) (Oxoid) and were stored at +4 °C.

Antifungal activity test

The antifungal activity of the extracts was carried out by disc diffusion test (18) using 100 µl of suspension containing 10^8 CFU/ml of fungi spread on SDA medium. Sterile 6 mm diameter filter paper discs were impregnated with 300 µg all the steril test material and placed onto SDA. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ofloxacin (5µg/disc), sulbactam (30µg) + cefoperazona (75 µg) (105 µg/disc) and/or netilmicin (30µg/disc) were used as positive reference standards to determine the sensitivity of one strain in each fungi species tested. The inoculated plates with fungi were incubated at 36 °C for 24–48 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its antifungal activity. Five disc per plate and three plates were used, and each test was run in triplicate.

Microdilution assays

The minimal inhibition concentration (MIC) values were also studied for the fungi which were determined as sensitive to the extracts in disc diffusion assay. The inocula of fungi were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Green tea extracts dissolved in methanol were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 7.80 to 500 µg/ml in 10 ml sterile test tubes containing nutrient broth. MIC values of green tea extracts against fungi strains were determined based on a micro-well dilution method (19). The 96-well plates were prepared by dispensing into each well 95 µl of SDA and 5 µl of the inoculum. A 100 µl from green tea extracts initially prepared at the concentration of 500 µg / ml was added into the first wells. Then, 100 µl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 µl of nutrient broth without compound and 5 µl of the inoculum on each strip was used as negative control. The final volume in each well was 200 µl. Maxipime (Bristol-Myers Squibb) at the concentration range of 500-7.8 µg /ml was prepared in SDA and used as standard drug for positive control. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24–48 h. Microbial growth was determined by absorbance at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, Vermont, USA) and confirmed by plating 5 µl samples from clear wells on SDA medium. The extract tested in this study was screened three times against each organism. The MIC of each extracts was taken as the lowest concentration that showed no growth.

Determination of catechins in tea leaves

Green (Fresh) tea flush (two leaves and one bud) harvested from seed propagated tea plants (*Camellia sinensis*) from Ikizdere-Rize region at 3 main harvest period (May, July, September) in 2008 year. The catechin-based flavonoids

Table 1. Antifungal activity of green tea extracts against *Candida* species

Species	1st harvest				2nd harvest				3rd harvest			
			MIC values				MIC values				MIC values	
	Inhibition zone (mm in diameter)	Positive control standard disc	Extract	Maxipime	Inhibition zone (mm in diameter)	Positive control standard disc	Extract	Maxipime	Inhibition zone (mm in diameter)	Positive control standard disc	Extract	Maxipime
Candida albicans	16	19 (OFX)	125µl/ml	7.81µl/ml	16	19 (OFX)	250µl/ml	125µl/ml	16	18 (NET)	62.5µl/ml	125µl/ml
Candida glabrata	15	18 (NET)	250µl/ml	125µl/ml	14	19 (OFX)	62.5µl/ml	7.81µl/ml	15	17 (OFX)	62.5µl/ml	7.81µl/ml
Candida kruseii	14	21 (OFX)	125µl/ml	31.3µl/ml	15	17 (NET)	125µl/ml	62.5µl/ml	16	19 (OFX)	62.5µl/ml	31.50µl/ml
Candida parapsilosis	13	17 (OFX)	62.5µl/ml	7.81µl/ml	15	16 (OFX)	125µl/ml	125µl/ml	16	19 (OFX)	62.5µl/ml	125µl/ml
Candida dubliensis	12	14 (SCF)	125µl/ml	62.5µl/ml	13	14 (OFX)	125µl/ml	7.81µl/ml	13	20 (SCF)	31.50µl/ml	7.81µl/ml

*OFX, ofloxacin (5 µg/disc); SCF, sulbactam (30 µg) + cefoperazona (75µg) (105 µg/disc); NET, netilmicin (30 µg/disc) were used as positive reference standarts antibiotic discs (oxoid).

Table 2. Catchine content (dry weight %) of tea leaves

Catechine group	Catechine content (%)			
	1st Harvest	2nd Harvest	3rd Harvest	Average
EGCG	10.60b	11.83ab	12.06a	11.50
EGC	5.74	5.99	6.17^{NS}	5.97
ECG	1.55c	1.62b	1.87a	1.68
EC	0.88b	0.93ab	1.12a	0.98

*Values in the same line with different lower-case letters are significantly different at $P<0.05$.

such as epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epicatechin (EC) were determined by HPLC method (20).

Statistical analysis

The experiment was a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan multiple range test at $P<0.05$ significant level.

RESULTS AND DISCUSSION

Antifungal activity of tea leaves against Candida

The paper describes the seasonal antifungal activity of crude methanol extracts of green tea against 5 *Candida* species and also determined the catechin-based flavonoids in green tea leaves such as epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epicatechin (EC). The results of the seasonal antifungal activity of the investigated extract are shown in Table 1 and catechin-based flavonoids are shown in Table 2.

The most pronounced activity with inhibition zones of more than 17 mm was shown by the methanolic extracts of green tea at 3rd harvest season against to *Candida albicans*, and followed by *Candida kruseii* (16 mm) at 3rd harvest and *Candida parapsilosis* (16 mm) at 3rd harvest. Whereas the extract of the green tea plants exhibited the lowest activity against to *Candida dubliensis* (12 mm in 1st harvest and 13 mm in 2nd and 3rd harvests). Control treatments did not show any activity (Table 1).

Previously, Chou et al. (17) showed that the antimicrobial activity of oolong tea varied among harvest season. The results obtained in the course of the present study are in agreement to a certain degree with the traditional uses of green tea evaluated. *Camellia sinensis* seems to be valuable sources for antifungal drugs, especially against *Candida albicans*.

Catechin composition of tea leaves

Catechin-based flavonoids in green tea leaves) were shown in Table 2. Catechin analysis has shown that tea leaves studied contained four major compounds such as epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epicatechin (EC) (Table 2).

There were statistically differences between harvest times on all catechin groups ($p<0.05$), except epigallocatechin (EGC) (Table 2). Epigallocatechin-3-gallate (EGCG) acid was the dominant catechin group (10.60–12.06%) in tea leaves and followed by epigallocatechin (EGC) (5.74–6.17%), epicatechin-3-gallate (ECG) (1.55–1.87%) and epicatechin (EC) (0.88–1.12%), respectively (Table 2). In our experiment total catechins are found to be around 21% (Table 2). Previously catechin content of green tea in Taiwan found as 17% (21). Among different tea products, green tea has the highest content catechins because its amount decrease parallel to fermentation. Catechins have contributed taste, aroma, astringency and color in tea. Chou and Lin (22) showed that tea flush including catechins had strong antimicrobial activity. Hirasawa and Takada (23) and Sitheeque et al., (24) showed antifungal activity of both green and black tea catechins against *Candida albicans*. Hara et al. (25) also revealed antimicrobial activity of tea

catechins including EGC, EC, EGCG, and ECG against the growth of *Clostridium botulinum*. ECG and EGCG, have been reported to be powerful antagonists of human immunodeficiency virus reverse transcriptase (26). Based on these results, it is possible to conclude that green tea has strong and broader spectrum of antifungal activity against *Candida* group fungi and antifungal activity shown by tea extracts is probably due mainly to the catechin EGCG and perhaps EGC. The contributions of the other catechins are limited by the fact that only small amounts are presented. A study conducted in Japan reported that drinking green tea reduced the incidence of dental caries among school children (27) due to an increased intake of fluoride, but polyphenol moiety of tea was thought to be responsible (28). Elvis-Lewis and Steelman (29) stated that drinking more tea improved dental health among school children in USA. It is also patented that tea catechins may have some commercial usefulness in the general field of mouth hygiene (30).

As a conclusion of this study, it can be said that the antifungal activity of green tea leaves may be closely correlated to catechin content.

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