# Study the Inhibitory Effect of Green Tea Extracts on Growth of Some Dermatophytes

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## Abstract

The antifungal profiles of green tea (*Camellia sinensis*) were examined against clinical isolates of Dermatophytes (*Trichophyton mentagrophytes*, *T.verrucosum* and *T.rubrum*) and some pathogenic yeasts (*Candida albicans* and *Cryptococcus neoformans*). Maceration method was used for the extraction of active substances from the green tea with cold and hot ethanolic and water extraction solvents. Agar dilution method was used in the antifungal susceptibility studies. This study revealed that the cold ethanolic extract was the most effective one, followed by the hot ethanolic extract while the aqueous extract was the least effective against all the tested fungi. All the extracts exhibited greater antifungal activity against Dermatophytes than the yeasts. The highest inhibitory effect of cold ethanolic extract reached to 96.92% at 20 mg/ml concentrations for *T.mentagrophytes* and *T.rubrum*, and 92.30% for *T.verrucosum*. In yeasts, the rate of inhibitory effect showed that with 200 mg/ml of cold ethanolic extract, the inhibitory rate of *C.albicans* and *C.neoformans* were 83% and 84% respectively.

This study, therefore, suggest that the green tea could have strong biocidal substance against Dermatophytes and some pathogenic yeasts, and therefore, may have the potential effective role in the treatment of human and animal dermatophytosis when used as ointment preparation.

# دراسة التأثير التثبيطي لمستخلصات الشاي الأخضر على نمو بعض الفطريات الجلدية

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## الخلاصة

أجري اختبار تأثير الشاي الأخضر من نوع *Camellia sinensis* كمضاد فطري لبعض الفطريات الجلدية مثل*Trichophyton mentagrophyte, Trichophyton verrucosum, Trichophyton rubrum* كذلك ضد بعض أنواع الخمائر الممرضة مثل *Candida albicans, Cryptococcus neoformans* حيث استخدمت طريقة تنقيع أوراق الشاي الأخضر بالكحول البارد والحار وكذلك الماء لغرض استخلاص المادة الفعالة منه. ودرست حساسية الشاي الأخضر كمضاد فطري من خلال تخفيف الوسط الزرعي بالمستخلصات المستخدمة. أظهرت هذه الدراسة ان المستخلص الكحولي البارد كان الأكثر فعالية ضد الفطريات المذكورة ثم يليه المستخلص الكحولي الحار أما المستخلص المائي فكان الأقل تأثيرا. لوحظ ان أعلى نسبة تثبيط للمستخلص الكحولي البارد لكل من*T. mentagrophyte* و*T.rubrum* كانت% 96.92 عند تركيز 20 ملغم/مل أما فطر *T.verrucosum* فقد وصلت نسبة التثبيط إلى 92.3% أما بالنسبة لخميرتي مثل*Candida albicans*

و *Cryptococcus neoformans* فقد لوحظ ان نسبة التثبيط كانت83% و84% على التوالي عند تركيز 200ملغم/مل للمستخلص الكحولي البارد ولهذا اقترحت هذه الدراسة استخدام الشاي الأخضر لاحتوائه على مواد ذات فعالية بايولوجية قوية ضد الفطريات الجلدية وبعض الخمائر الممرضة وبالتالي إمكانية استخدامها كمراهم في علاج الإصابات الفطرية الجلدية في كل من الإنسان والحيوان.

## Introduction

The use of plants for medicinal purposes permits the introduction of antibiotic and other modern drugs (1). The potency of herbal remedies soon became an issue of dispute due to lack of qualitative identification of their bioactive components (2). The sreach for more potent chemotherapeutic agents led to discovery and development of antibiotics (3). However, as years passed several microorganisms developed resistance, to these antibiotics thereby rendering them important and otherwise useless (4).

Overtime, the economy of producing these antibiotics and subsequent cost of acquiring medications was fast getting out of the reach of common man. In recent time, some of these antibiotics have been found to exhibit neurotoxic effects while a few others cause severe liver damage and bone marrow depression. All these factors led to the re-birth of intensive search for natural products from plants which contain active ingredients of medicinal values.

Green tea considered one of these important plants, It is originated from China and other Asian Countries which mainly produced from *Camellia sinensis* var *sinensis* has a too high content of polyphenols. Also it has been considered a medicine and healthful beverage since ancient time. This type of tea is produced by drying and steaming of fresh leaves to inactivate polyphenol oxidase and thus, non-oxidation occur (5).

However, it contains the catachines which have a wide range of strong antioxidants potential and posses antimutagenic, anticarcinogenic, antidiabetic and anti-inflammatory properties (6,7,8). In addition, the miniral compounds such as Floride and manganese are risponsible for digestive tract function while the organic compound affects on activity of vision, skin, and cardiovascular system (9).

Further more, green tea leaves contain two main components which act upon humans health: Caffeine and Theophylline, the first one acts mainly upon the central nervous system stimulant and the second one induces vasodilator and bronchodilator effects and a much higher diuretic effect than caffeine (10).

Large number of studies revealed that green tea catechine have antibacterial and antiviral activity by its effectiveness against any type of diarrhea and typhoid, also it inhibits the reproduction and growth of many bacteria, which some types of *Salmonella*, *Clostridium*, *Bacillus*, *Helicobacter pylori*, and *Staphylococcus aureus* (11). Regarding its antiviral action, it affect against the Influenza virus, especially in its earliest stage, as well as against Herpes simplex virus and Adenoviral infection. But the studies on its effect as antimycotic infection particularly antidermatophytic infections are very rare (12,13). Despite of wide-spreading of dermatophytosis which results in huge economical loss in animal products, treatment cost, in addition to increase rate of human infection, so the aim of this study is to demonstrate the capacity of many types of green tea extracts to kill or inhibit some types of dermatophytes and some pathogenic yeasts by comparision its effect with antimycotic.

## Materials and Methods

**- Organisms:** The fungi used in this study were *T.mentagrophytes*, *T.verrucosum*, *T.rubrum*, *Candida albicans*, and *Cryptococcus neoformans*, isolated from patients with Dermatophytosis and diagnosed in Department of Microbiology, College of Veterinary Medicine .These strains were identified based on colony and microscopic morphology,

urease test, hair perforation test, germ tube test and ability to pigment production on Corn Meal Agar (CMA) plus 2% dextrose.

**- Preparation of green tea extracts:**

1. **Preparation of cold ethanolic extract:** Green tea leaves were obtained after inported from other country then pulverized into fine powder by using electric blender. Extraction was done with cold ethanol. Forty grams of powdered sample were added to 250 ml of 70% ethanol in flask for 24 hrs on magnetic stirrer in room temperature then the mixture was precipitated by centrifuge at 3000 rpm/15 min, after that the supernatant was collected and further filtered through filter paper Wattman No.1, the filtrate were evaporated to semi-solid mass. The dry extract were later reconstituted with their respective extractant (ethanol) to give a concentration of 200 mg/ml for antimicrobial activity which considered as a stock solution of extract which filtrated through 0.22 µm Millipore and different concentrations of extracts(20, 10 and 5 mg/ml) were carried out to study the effectiveness of extract against Dermatophytes with plate ofSabouraud dextrose agar (SDA) free extract as control (14).
2. **Preparation of hot ethanolic extract**: This type of extract was carried out by taking 40 gm of powder and put in thumble of soxhlet apparatus. Then 250 ml of 70% ethanol was added to the extracted flask and the process was continued for 3 hrs at 60 ºC then the mixture was filtered through Wattman No.1 . The other steps was similar to cold ethanolic extract (15).
3. **Preparation of aqueous extract**: For aqueous extract preparation, the same steps in the preparation of cold ethanolic extract was followed except of using distilled water instead of alcohol.

The radial growth was measured, and the rate of growth inhibition was calculated by using specific ruler(16).

- **Effect of extracts on some yeasts:**

The agar-well diffusion method was used (17) The agar plates wells were inoculated with 20 µL of yeast suspension that contain ($1\times 10^8$ yeast/ml) via sterile swabs and it left at room temperature for 30 – 60 min to dry. Plugs were made at 6 mm by sterile cork porer, then different concentrations of extracts were prepared (200, 100, 50, 25 and 10 mg/ml) and were added 0.1 ml of each of them in each well with sterile distilled water in one of the well as control. After that the plates were inoculated at 37 ºC for 24-48 hrs. The diameter of clear zone of inhibition was measured.

- **Preparation of 1% clotrimazol as standared control:**

Clotrimazol was used in this study to compare its effect against fungi with green tea extract effects. This was done by dissolving 50 mg of clotrimazol in 5 ml of organic dissolvent (Dimethyl sulphoxide 100%) to obtain of final concentration of 10 mg/ml (17).

## Results

Effect of green tea extracts on the radial growth of some strains of dermatophytes are listed in (Tables 1, 2, 3), illustrated in (Fig 1), and the effect of these extracts on some pathogenic yeasts as zones of inhibition are listed in (Tables 4, 5).

This study found that the cold ethanolic extract was the most effective one, followed by the hot ethanolic extract while the aqueous extract was the least effective against all the test fungi.

Generally, there was a marked reduction in viability of all the test fungi with increased concentration. There was 96.92% loss of viability of *T.mentagrophytes*, *T.rubrum* and 92.30% of *T.verrucosum* at 20 mg/ml concentration of cold ethanolic extract when compared with standard antifungal (clotrimazol), and 83% and 84% of *C.*

*albicans* and *C.neoformans* at 200 mg/ml of cold ethanolic extract. Furthermore, it was shown that the *T.rubrum* ranked as the highest susceptibility against all types of green tea extracts, followed by *T.mentagrophytes* and the *T.verrucosum* ranked as the lowest effect degree through dermatophytes.

**Table (1) Effect of cold ethanolic extract of green tea on the growth of some fungal isolates colony (mm)**

| Conc. (mg/ml) | Mean of diameter of fungal growth(mm) | | |
|---|---|---|---|
| | ***T.mentagrophytes*** | ***T.verrucosum*** | ***T.rubrum*** |
| **0 (Control)** | 65 | 19 | 73 |
| 5 | 60 | 14 | 30 |
| 10 | 50 | 10 | 20 |
| 20 | 2 | 5 | 2 |
| 1.**25(Clotrimazol)** | 0 | 2 | 1 |

**Table (2) Effect of hot ethanolic extract of green tea on the growth of some fungal isolates colony (mm)**

| Conc. (mg/ml) | Mean of diameter of fungal growth(mm) | | |
|---|---|---|---|
| | ***T.mentagrophytes*** | ***T.verrucosum*** | ***T.rubrum*** |
| 0 (Control) | 65 | 19 | 73 |
| 5 | 64 | 17 | 50 |
| 10 | 59 | 14 | 38 |
| 20 | 15 | 10 | 4 |
| 1.25(Clotrimazol) | 0 | 2 | 1 |

**Table (3) Effect of aqueous extract of green tea on the growth of some fungal isolates colony (mm)**

| Conc. (mg/ml) | Mean of diameter of fungal growth(mm) | | |
|---|---|---|---|
| | ***T.mentagrophytes*** | ***T.verrucosum*** | ***T.rubrum*** |
| 0(Control) | 65 | 19 | 73 |
| 5 | 65 | 19 | 65 |
| 10 | 60 | 15 | 60 |
| 20 | 20 | 13 | 18 |
| 1.25(Clotrimazol) | 0 | 2 | 1 |

**Table (4) Effect of different extract concentrations on the growth inhibition of *Candida albicans* (mm)**

| Conc. (mg/ml) | Mean of diameter of fungal colony(mm) | | |
|---|---|---|---|
| | **Cold ethanolic extract** | **Hot ethanolic extract** | **Aqueous extract** |
| 0(Control) | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 |
| 50 | 11 | 2 | 0 |
| 100 | 15 | 12 | 2 |
| 200 | 17 | 15 | 10 |

**Table (5) Effect of different extract concentrations on the diameter inhibition of *C.neoformans* (mm)**

| Conc. (mg/ml) | Mean of diameter inhibition of extract(mm) | | |
|---|---|---|---|
| | **Cold ethanolic extract** | **Hot ethanolic extract** | **Aqueous extract** |
| 0(Control) | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 |
| 50 | 10 | 0 | 0 |
| 100 | 13 | 11 | 6 |
| 200 | 16 | 15 | 10 |

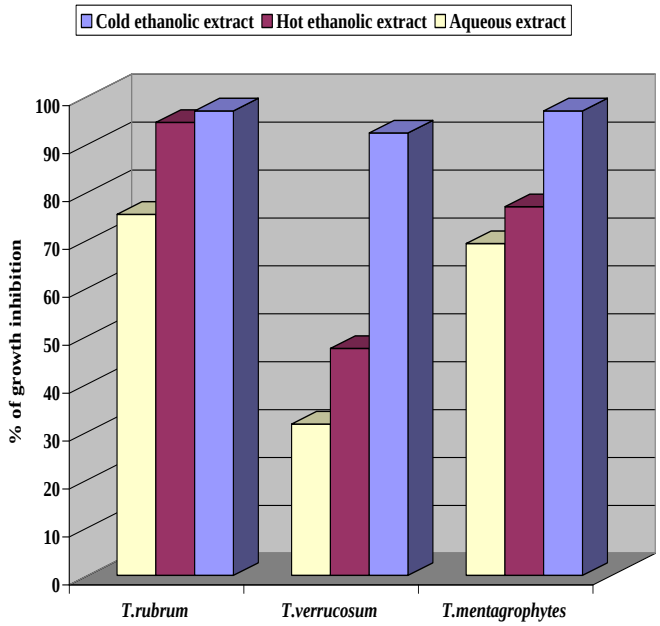


**Fig (1) Effect of Green tea extracts at 20 mg/ml on some strains of dermatophytes**

## Discussion

Tea is the most consumed drink in the world after water. Green tea has been represented a healthful beverage since ancient time (18) mentioned the chemical composition of green tea is complex: protein (15-20% dry weight), aminoacid (1-4% dry weight), carbohydrates (5-7% dry weight), lipid, vitamins (B, C, E), pigment, volatile compound, mineral and trace elements (5% dry weight) .But the most intresting group of this type of tea leaf components is polyphenols. So, this tea can be considered as an important dietary source of polyphenols, particularly flavonoids. These flavonoids are phenol derivatives synthesized in substantial amount (0.5-1.5%) as studied by (19).

Many studies conducted over the last 20 years have shown that the green tea plyphenolic catechine, in particular (-)-epigallocatechin-3-gallate (EGCG) represent approximately 59% of total catechines,(-)-epigallocatechin (EGC) 19%, (-)-epicatechin-3-gallate (ECG) 13.6% and (-)-epicatechin (EC) 6.4%. This study shows the inhibitory effect of green tea extracts on some strains of dermatophytes (*Trichophyton* spp.) due to the catechin attached the cell membrane and caused lysis of the conidia and hyphae, these findings agreed with (20) and (21) when they used electron microscopy.

In contrast with (22) who reported that 2.5% of Black tea extract completely inhibited the growth of *T.mentagrophytes* and *T.rubrum*. However, even at 10% concentration, this extract did not inhibit the growth of *C. albicans* or *C.neoformans*.

In this study demonstrated the inhibitory effect of green tea extracts suppress the growth of moulds and yeasts but to different extent depending on extract concentrations, and the fungicidal effect could be due to EGCG, EGC and GC.

Although (23) highlighted that the EGCG could inhibit ergosterol synthesis by disturbing folic acid metabolism in *Candida albicans* which have been proved by (24).

In the present study, the cold ethanolic extract exerted the greatest inhibitory activity against the tested fungi followed by hot ethanolic extract, while aqueous extract exhibited the least, and this due to activity of ethanol to dissolve multivariable compounds either polar or non-polar as mentioned by (25) which may be responsible for the greater antifungal efficacy than water.

The inhibitory effect of tea depends upon type of tea, preparation of the extracts method and its concentration and tested microbes, However, the biological activity of the tea increased with high concentration of extract.

In a study to (26) who record the sensitivity of 10 different wood-rotting fungi towards eight samples of tea and two samples of coffee, they discovered that the green tea caused the maximum growth inhibition and it was 100% in case of *Phanerochaete chrysosporium* and *Sporotrichum pulverulentum*While the effect of hot ethanolic extract could be due to thermostability of some bioactive chemical constituents which might have been enhanced by the possibility of an increase in the solubility of active ingredients of plant material in hot alcohol making more constituents available in the resulting extract.

However, this work has shown that ethanol is the extractant of choice because the bioactive substances in green tea tested are less soluble in water than in ethanol.

Therefore, using appropriate extractants could be purified and manufactured as antiseptic agent (as ointment) for the treatment of skin infections caused by these groups of fungi. Also these extracts could be combined with antimycotics that may be beneficial and may contribute and increase the effective medical treatment of these fungi.

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