

Catechin extraction and purification of green tea (*Camellia sinensis*) and using it in treatment the *candida albicans*

Y.D.Al-asadiy

Department of Biology , College of science ,Univ.Almuthana -Iraq

Abstract

The extraction and purification Catechin of green tea *Camellia sinensis* using Separation of retail, as the extraction first with hot water and then hash extracted in two stages first mixed with chloroform and after were obtained at the layer of water, then the second phase mixed with acetate ethyl get disposal from compounds non polar was obtained a brown color dark without viscosity of a substance Catechin has been diagnosed and confirmed using reagents chemicals as well as technical thin layer (TLC) and the amount of Relative movement to the compound (0.76) when using the solution separated acetate ethyl: Benzene (9:11) using the technique High Performance Liquid Chromatography(HPLC) have emerged as one package. in the other side applied the catchin as anti mycotic agent, The susceptibility of *Candida albicans* to catechin were evaluated. The concentrations of catchin causing growth inhibition of tested strains of *C. albicans* in different zone inhibition .

Keywords: Green tea , Catechin, TLC, HPLC, ,*candida albicans*.

Introduction

The tea one of the most drinks popular in the world and different from the commercial distribution of consumption of tea types, as the consumption of green tea and black, about 20% and 78% of production respectively (1), because black tea, green tea leaf of the same *Camellia sinensis*, but they differ in the production process as the influence of tea in its content of phenols multi After picking leaves young wilt, and damage to rolled and fermentation full and baked and dried to prepare black tea, while offering securities for steam to discourage the effectiveness of the enzyme polyphenol oxidase (PPO) then dried to prepare green tea. The third type Oolong tea, prepared after compress and fermenting partially dried (2). Serve green tea to increase the ability to fight disease and reduce cholesterol level and keeps the blood clotting and resisting a stroke and contains green tea a good percentage of the mineral fluorine resistant to tooth caries and fight cancer, preventing the growth of blood vessels that supply these tumors them and help them to survive and grow, and is characterized also effect the anti-oxidant of any maintenance of the cells from damaging substances and helps to reduce high blood pressure, as it leads to relaxation of the muscles controlling the degree of capture of the arteries, It also has effective against pathogenic bacteria (3). containing tea leaves totals chemical group , part of them totals effective vital is to alkaloids purine, which include: caffeine, which has a rate ranging between 5.2 to 5.4% of dry weight (4), and the three-Triterpene, and Flavanols representing Catechins the President where, in addition to that contains green tea on the minerals and vitamins

such as fluoride, potassium, aluminum and vitamin C and vitamin A and vitamin E , play phenols multiple tea an important role in many events critical, it was found that Catechins of green tea and EGCG effectively to offset the free radicals will increase by 100 and 25 times for effective antioxidants of vitamins C and E, respectively (5). *Candida albicans* is part of the indigenous microbial flora in humans and can be found in the oral cavity and the digestive and vaginal tracts, and is unique among opportunistic pathogens because it is part of the normal microbial flora of the host(6). However, an increased prevalence of candidosis is well documented and has been attributed to the widespread use of antibiotics and immunosuppressive agents(7). *C. albicans* has been shown to play an important role in oral candidosis, denture stomatitis and severe periodontitis(6). Therefore, a non-antibiotic agent that is both highly effective and safe might be important for the eradication of both antibiotic-susceptible and -resistant strains of *C. albicans*. There are several reports that show antifungal activity by natural products(8). Green tea is a natural substance that is commonly drunk worldwide, especially in Asia. Catechin from tea has been reported to have an antimicrobial effect against oral (9), intestinal(10) and food-borne1 bacteria, antitoxicity against various bacterial haemolysins(11) and antiviral activity(12).

Candida albicans has several properties which allow it to colonize and invade host tissues, often resisting eradication. Two of these properties, adherence and acid proteinase production, seem to be genuine factors. Phenotypic switching and molecular mimicry may also provide the organism with an arsenal of mechanisms, present in the mucous membranes of the mouth, intestinal tract, and vagina of healthy people. Under certain circumstances, it may cause superficial infections of the skin, mouth, or vagina. Infection of the esophagus and severe invasive systemic infections may occur in persons with human immunodeficiency virus(13)

The present study aims to extract and purify the material Catechin of green tea, which constitute a high proportion of its components and examined these extract (catechin) on the *C. albicans* as antimycotic agent .

Materials and methods

- Obtained a green tea from the local markets in powder of dried leaves stored in the bags packed, this type of green tea Chinese origin and has filled in Syria - Damascus.
- The composite index Catechin Standard has been obtained from the Company (Sigma).
- The preparation of hot water extract

Attended the melt (50) grams of the powder plant in the (500) ml of distilled water boiling temperature (100) ° C and left ten minutes, then filtrate by filter papers. Then completed the steps involved in the previous paragraph to get the sterile extract (14), which was then used in the preparation of the foundation solution dissolved in distilled water and the followers of the same steps to prepare the extract solution is the foundation of the cold water.

- Separation of compounds effective of catechin compound

Extracted and separated the active compounds the main (catchiness) from the leaves of green tea according to the method described by (3). Which included two phases, in the first stage mixing 200 grams of green tea leaves dried with 500 ml of distilled water, then put in the degree water bath 50 m for a period of four hours, then ran the mixture using a filter paper .

The second phase include the purification of extracted concentrated from is the non-polar addition of chloroform to the mixture in a separating funnel by 1:1 (volume of extract: the size of chloroform), and mixing the mixture well and leave for an hour for the separation of phases as a phase upper layer of water, and the phase of the lower back of the layer chloroform. Then transferred to the upper layer to class vessel clean, has been eliminated from the lower layer (chloroform layer), was purified and the top layer of low-lying polar compounds by adding ethyl acetate to layer in a separating funnel by 1:1 (volume of extract: the volume of ethyl acetate). Mixing and mix well and leave for 24 hours for the separation of phases as a phase upper layer of water, and phase lower due to layer acetate ethyl, then disposed of the top layer (layer acetate ethyl) and the collected lower layer (Sample layer) has been vaporization him with rotary evaporator degree temperature to 50 m in order to focus to a third of its original size. Was dried using Lyophilizer to get dark brown powder.

- Detection of chemical compound (Catechin)

Was conducted in accordance with the following reagents to the extent stated in (14) is:

- Phenolic compounds
- Tannin compounds
- Flavonoids compounds
- Turbines and steroids compounds

- Detected of a compound Catechin using a technique Thin Layer Chromatography

Followed the way described (15) method sheets Thin layer chromatography (TLC) and the plates is a thin layer of aluminum coated with a layer of silica gel dimensions (20x20) cm and a thickness of (0.2) mm and processed from a company (Fluka) container material when exposed to ultraviolet light source. Put down the plate specimen in the form of small patches using capillary tubes and the use of two types of separated systems:

A. Ethyl acetate. Benzene by 11: 9

B. Acetic acid: chloroform ratio of 1: 9

Then examined the source of the plates under ultraviolet light at a wavelength (280) nm. Calculated Rf value of the separated spots for tow solution according the equation:

Distance made by sample

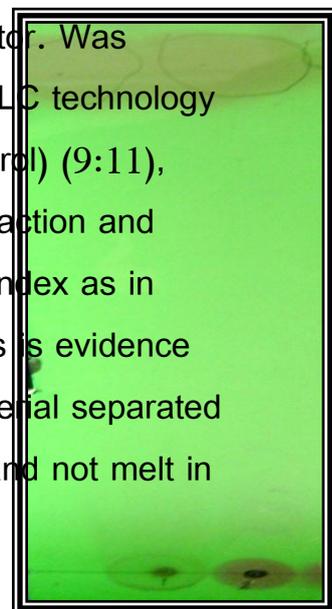
$$R_f = \frac{\text{Distance made by sample}}{\text{Distance made by solvent}}$$

Microorganism and culture condition:

All the isolates *C. albicans* obtained from three different hospitals in Al-Muthanna province (Al-Hussein Teaching Hospital, Al-Rumaitha General Hospital, Samawa Feminine Hospital) and *C. albicans* was cultured on Sabouraud agar. The plates were incubated aerobically at 37°C.

Results and discussion

Catechin compound separated from the rest of the compound in two phases using a separate retail (liquid – liquid). It adopted the system of distribution primarily on differences in the degree of polarization between the compound to be separated, especially that the degree of polarization is the main factor that controls the degree of melting system of distribution depends on the degree of melting compounds form between asking different polarity, when mixing the sample with chloroform and distilled water in this experience for the purpose of separating non-polar compounds from polar compounds where the compound is polar Catechin (3). Will be the outcome of chloroform all the non-polar compounds or remote degree of polarity of a compound Catechin the water will dissolve the compound Catechin compounds and some of it is very close polarity or similar to it. The second phase included the mixing of the form obtained in the first phase with ethyl acetate to purify the compound also Catechin compounds with non-polar or polar lows. Has been getting a brown color too dark devoid of viscosity after the concentration of the layer of water to your rotary evaporator. Was detected for the primer material obtained (dark brown) using the TLC technology and the use of two types of separation systems (ethyl acetate: petrol) (9:11), (acetic acid: chloroform) (9:1). He found that material it steps extraction and distance relative migration (R_f) 0.76 It is identical to a composite index as in Figure (1) It has been the emergence of spot dark in both systems is evidence that they compound Catechin, according by (15). proved this material separated from the plant green tea is its ability to melt in alcohol and water and not melt in



the acetate ethyl and methyl chloroform, and proved this article their ability to interact with the ferric chloride color blue green.



1- Standard material

2- purified material

Figure (1) detection of a composition primer Catechin (TLC)

- Detection chemical of Catechin compound

After obtaining the result of purification, containing the first part, adopted these tests to determine the nature of groups active in the material purified such as: (phenols multiple turbines, which included the tannins, and Flavonoids) so conducted many of the discoveries of chemical quality of these compounds in the two parts and the results were positive for the layer Water for the statements of phenols and tannins and Flavonoids, but for the turbines and the steroids have given negative results.

- High Performance Liquid Chromatography

Was to know the degree of purity using a technique high performance liquid (HPLC), where the recovered components extracted from column C-18 (0.46 × 25cm) 5µm to chromatography liquid high efficiency using a mobile phase and consisting of Acetonitrite and acetic acid and by (30:70) for 0-20 minutes the appearance of one peak and one for composite Catechin purified, indicating the absence of other compounds with him and at the time of the emergence of a composite Catechin purified at the minute (6.996) is identical to the time of the emergence of the composite index of composite Catechin at the minute (7.196), as shown in figure (2) and figure (3). Where the ratio of purified compound (100%) and the ratio was identical to the rate of the composite index. This result confirms previous statements purity (Catechin) to detect confirmation TLC and detected using a specific optical spectroscopy and we note that (Catechin) was purified to a

high degree of purity compared with the composite index. This is because the wide range of variation in the concentration of various phenols (Catechins) to several factors including: climate, season and pick out the cards, and agricultural practices of the plant and gardening, as well as the type of plant and age (16).

It has been reported that tea catechins have antibacterial activity against various pathogenic bacteria(17,18) Concerning fungi, (19) reported that 2.5% of black tea extract completely inhibited the growth of *Trichophyton mentagrophytes* and *Trichophyton rubrum*; however, even at a 10% concentration, this extract did not inhibit the growth of *C. albicans* or *Cryptococcus (Filobasidiella) neoformans*. Recently, botanical,(20) marine(21) and bacterial(22) natural products were reported to have antifungal activity. In the present study, we showed that the antifungal activity of catechins against *C. albicans*. For reference, a normal cup of tea has a concentration about 1000 p.p.m. polyphenol .three concentrations used in these experiment (1, 2,3 microgram/ml,) showing its effects in the figure (4) . (23) reported that the mechanism of the bactericidal effects of catechins primarily involved acting on and damaging bacterial membranes of *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activities of catechins were predominantly related to the gallic acid moiety and the hydroxyl group member(23). The mode of catechin action involves inducing rapid leakage of small molecules entrapped in the intraliposomal space and aggregation of the liposomes(23). (24) examined the mechanism of the effects of green tea catechin on *T. mentagrophytes* using electron microscopy and suggested that catechin attacked the cell membrane and caused lysis of the conidia and hyphae.

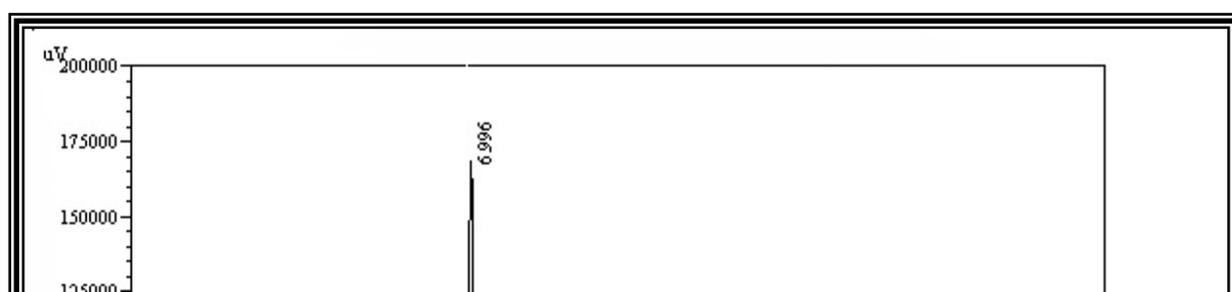


Figure (2) : Technology HPLC liquid high enough to measure the absorbency (Catechin) purified from green tea.

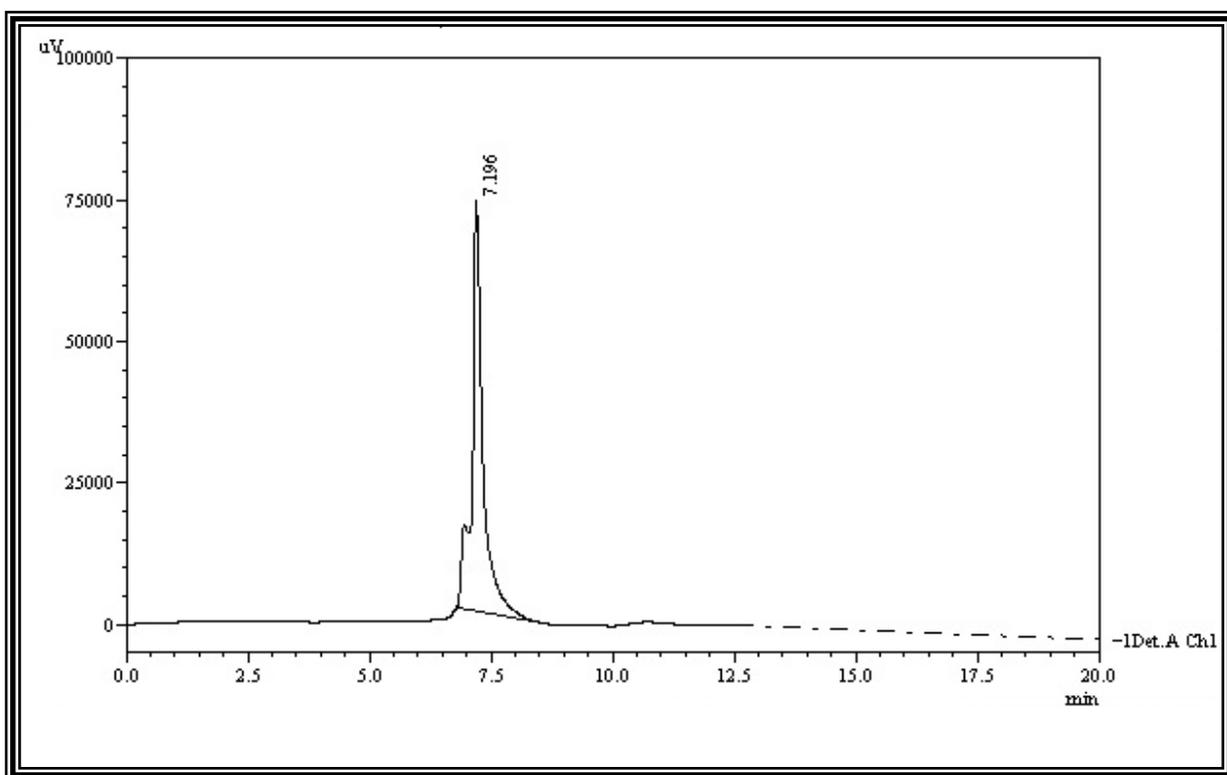


Figure (3) :Technology HPLC liquid high enough to measure the absorbency (Catechin) standard

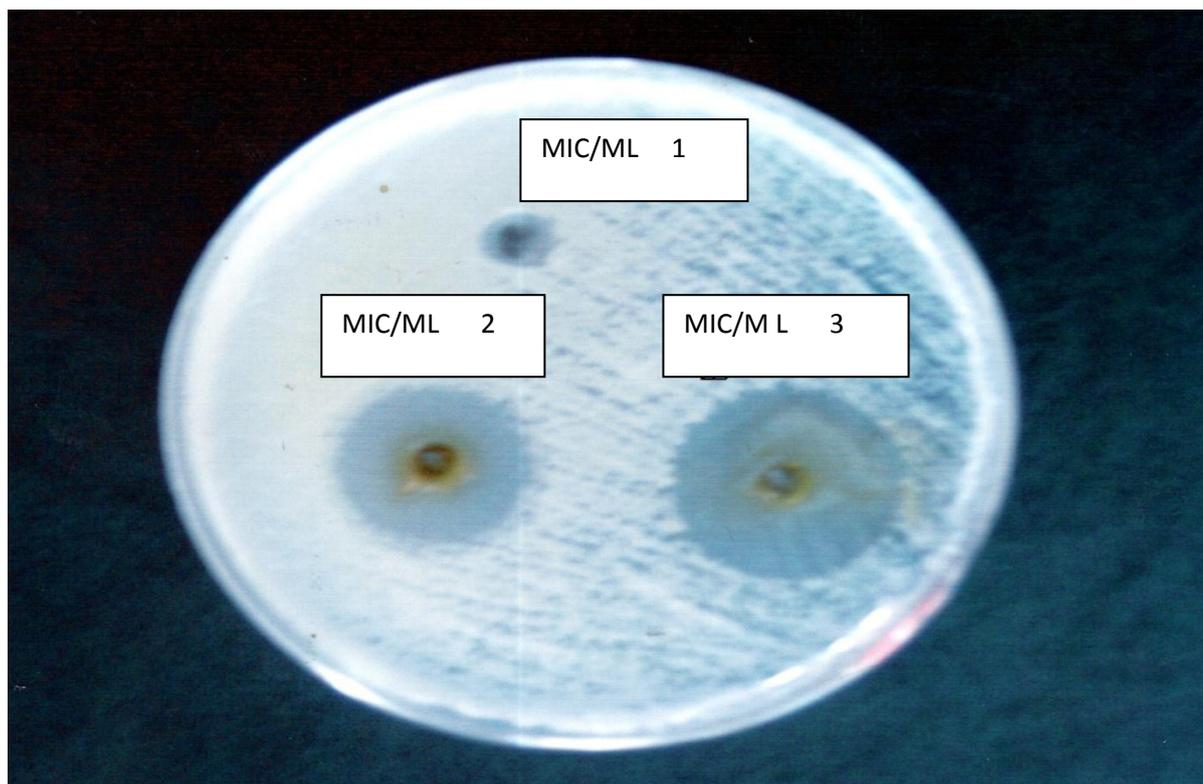


Figure (4): showed zone of inhibition for different concentrations of catechin (1,2 and 3 microgram /ml) on *C.albicans*.

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