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Investigation of antimicrobial effects of tea leaf extract on microorganisms in culture media

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Abstract

Regarding to the fact that using synthesis antimicrobial components in food stuffs has undesirable impacts including mutation, toxicological effects, and carcinogenesis in human body, they are being omitted from previously used antimicrobial components list; therefore, preparation and production of natural anti-bacterial components as a substitute seem to be necessary. Tea leaf has been recognized as a substance for antibacterial activity against the effect of microorganisms in the present research, antibacterial activity (paper-disc method), determination of the minimum inhibitor concentration and minimum microbe bactericide concentration (Agar and Broth Dilution Method) of tea water extract in three repetitions on *Staphylococcus aureus* (PTCC:1112), *Escherichia coli* (PTCC:1330) *Penicillium sp*. (PTCC:5251) and *Clostridium perfringenes* (PTCC: 1651) microorganisms were investigated. Based on the results of antimicrobial activity, *Staphylococcus aureus* showed the highest sensitivity and *Escherichia coli* had the highest resistance against tea water extract. Results of the minimum inhibitor concentration (MIC) and minimum bactericide (MBC) for Escherichia coli were determined to be 750 and 1500 mg/L and for microorganisms they were identified to be 375 and 750 mg/L respectively.

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Introduction

The interaction between microorganisms and plants and animals is a natural and permanent phenomenon. Since human's foods are mainly consisted of plants and animals or the products obtained from these sources, it can be easily perceived that our food resources can have microorganisms with interfering action with that substance (food). Occasionally, the interactions between organisms and our foods are useful for us and sometimes they are harmful and cause illness and food poisoning and or result in food stuffs decay. In order to recognize these actions and prevent them, factors inhibiting their growth must be identified the most main of which that is the presence of inhibitor materials or preventive dams (obstacles) can be mentioned (Mehrabian, 1995). Tea is used as a beverage in most parts of the world. Consumption of tea has a considerable effect on human's health respecting cholesterol decrease and also plays a protective role against cardiovascular diseases and cancer (Chou *et al.,* 1999). Most undesirable effects are resulted from consumption of tea in human and food products which are related to polyphenol compounds existing in tea extract due to their strong antioxidant property (Mir Ahmadi, 1999; Chou *et al.,* 1999). Abundant research has been performed on the antimicrobial effect of tea extract and the obtained results denote the existence of this property in disease making and decaying microorganisms in human and food stuffs (Ayaz *et al.,* 2008; Sasaki *et al.,* 2004). This study aimed to investigate the antibacterial effect of tea leaf extract planted (cultivated) in Iran since no considerable work has been done in this regard till the present time.

Materials and methods

Preparation method of tea extract

In order to obtain comprehensive study, tea leaves grown in Iran were prepared and purchased from different tea farmlands in the north of Iran including *Lahijan*, *Tonekabon*, *Ramsar*, and *Roudsar* and to prevent undesirable reactions they were quickly blanched and frozen.

Preparation method of tea leaf extract

25 g of tea leaves which have been previously mashed in a mixer is weighted precisely and after being transferred to 250 ml Erlenmeyer, an amount of 100 ml distilled 80 C° degree water is added to it and is maintained at the same temperature, and is then strained by a filter paper and after that, it is evaporated and purified under vacuum condition in a temperature less than 40 C° degree. After excluding water from the extract, it is dried in a rotary evaporator and finally transformed into powder (Mir Ahmadi, 1999).

Preparation method of microorganisms

All the microorganisms including Staphylococcus aureus (PTCC:1112), Escherichia coli (PTCC:1330) ,Clostridium perfrringenes (PTCC:1651), Penicillium sp. (PTCC:5251) were used. Bacteria were-cultivated on enriched culture medium for desirable activation (cooked meat, Blood Broth, EC Broth, cooked meat, respectively) then they were cultivated on a selective medium in a surface culture way in order to gain healthy and active young colonies. All microbial suspensions provided from the above-mentioned microbes have been prepared with control turbidity degree of 0.5 McFarland regarding the number of microorganisms; it means that the basis of this work with suspensions is based on the number of microorganisms equal to 1.5× 108/ml (Kumudavally *et al.,* 2008).

Method for determination of antimicrobial property- Filter paper disc diffusion Method

Using filter paper No 40, small discs are cut and sterilized. Then, the discs are emerged in the solution containing tea leaf extract with concentration of 3000 mg/L, after that, discs soaked in the solution are placed on the previously prepared agar Mauler Hinton and the intended bacteria of this test which had been thoroughly arcaded on it with determined interval. Subsequently, the petri-dish is heated inside the incubator with optimum temperature of the under-test bacteria for 24 hours and sometimes for

48 hours and after this time, discs surroundings are examined; zone of growth inhibition diameter is measured and reported (Sakanaka *et al.,* 2000). According to Filter paper disc diffusion method values for zone of growth inhibition of microbes regarding anti-microbial activity are determined as follows:

The zone smaller than 5.5 is considered inactive, 5.5-10 relatively active, 11-19 active, and equal or lager than 20 (Ayaz *et. Al.,* 2008).

Determination method for minimum inhibitor concentration (MIC)

First, a series of dilutions for tea leaf extract are prepared in a liquid medium such as Nutrient liquid; at the beginning concentration of 3000 mg/L is used preferably, and after that, the concentrations are become more diluted respectively (each time the dilution is become two times of the previous one) then, a determined amount of bacteria (equal to 0.5 McFarland) is added to each dilution and the tubes are put in the incubator with a temperature proportionate to the optimum growth of the investigated microorganisms for 24 hours; and after passing this time, some of this liquid culture medium is taken and is then cultured on Nutrient agar medium. The first tube test which has the least growth rate compared to the control pipe is recorded and its dilution and concentration are reported as the minimum inhibitor concentration of that microorganism (Imene *et al.,* 2009).

Determination method of minimum bactericide concentration

The method used here is similar to MIC so, after achieving MIC, a dilution of tea extract in which no colony is grown in the intended petri-dish after being heated in the oven for 24 hours in optimal growth temperature of that microorganism is considered as the minimum bactericide concentration and dilution (Imene *et al.,* 2009).

All the tests were repeated three times and compared to the control group. Results were evaluated as the mean of those three repetitions.

Results and discussion

In table (1), results obtained from zone of growth inhibition of tea leaf extract by paper disc in each three repetition after determining their mean and standard deviation and comparing them represented that the extract has an antimicrobial effect on all microorganisms and inhibition effect of the extract on warm (heated) microorganisms are determined to be positive especially *Staphylococcus aureus* showed the highest value. The least effect of extract related to heated gram-negative microorganism was reported to be *Escherichia coli*.

Results obtained from determination test for minimum inhibitor concentration (MIC) in table (2) show that the highest MIC has been 750 mg/L which relates to *Escherichia coli* and the minimum MIC for other microorganisms was determined to be 375 mg/L.

As it can be observed in table (3), the minimum bactericide concentration for *Escherichia coli* had the highest value i.e. 1500 mg/l and the index for other microorganisms this concentration were determined to be much less than 750 mg/l. however, *Escherichia coli* in concentration of 750 and other microorganisms in concentration of 375 mg/l had the growth value of less than 10 colonies.

According to all the previous researches tea leaf extract has an antimicrobial effect (Ishihara *et al.,* 2001; Kuchari *et al.,* 2006; Türkoğlu *et al.,* 2007; Sasaki *et al.,* 2004; Sokmen, *et al.,*2004; Stoicov *et al.,* 2009; Weiduo *et al.,* 2006) and the results gained for the Iranian tea leaf extract also conforms to the previous studies. Iranian tea comparing to the tea produced in other countries has lower mean of polyphenol, as the most main compound having antimicrobial effect (Mir Ahmadi, 1999). Therefore, regarding the zone of growth inhibition, the MIC and

MBC compared to the tea produced in other parts of the world, has a higher effective concentration.

Table 1. Values of Zone of growth inhibition of microorganisms examined by tea leaf extract.

Microorganisms	Penicillium sp	E.coli	CL. perfrringenes	St.Aureus
Zone of growth inhibition(mm)**	18±1.3	13±1.1	15.2±1	20.3±1.2

** Size of zone of growth inhibition less than 5.5 inactive, 5.5-10 relatively active, 11-19 active, larger or equal to 20 very active

Table 2. Minimum Inhibitor Concentration (MIC) of tea leaf extract in the investigated microorganisms.

Bacteria	Delusions				
	1/4	1/8	1/16	1/32	1/64
	750 mg/l	375 mg/l	187.5 mg/l	93.75 mg/l	46.875 mg/l
E.coli	-	+	+	+	+
St.Aureus	-	-	+	+	+
Penicillium Sp.	-	-	+	+	+
CL.perfringens	-	-	+	+	+

(+ means growth of microorganisms, - means growth inhibition of microorganisms)

Table 3. Minimum Bactericide Concentration(MBC)of tea leaf extract in the investigated microorganisms.

Bacteria	Delusions					
	1/2	1/4	1/8	1/16	1/32	1/64
	1500 mg/l	750 mg/l	375 mg/l	187.5 mg/l	93.75 mg/l	46.875 mg/l
E.coli	–	±	+	+	+	+
St.Aureus	–	–	±	+	+	+
Penicillium Sp.	–	–	±	+	+	+
CL.perfringens	–	–	±	+	+	+

(+ means growth of microorganisms, - means growth inhibition of microorganisms, ± means less than 10 growth colonies)

Results obtained for antimicrobial property of tea leaf by paper disc method showed that all samples had non-growth zone of growth inhibition diameter more than 10 mm, the effect of tea extract on all microorganisms regarding inhibition is classified into the set of active antimicrobial substances (Ayaz *et. al.,* 2008). The least non-growth zone of growth inhibition diameter relates to Escherichia coli gram-negative microorganisms which can be resulted from the wall of negative microorganisms; since the most effective antimicrobial factors of tea extract are phenols which are completely solvable in water and because microbes have a wall made of lipids, therefore, such extract cannot have inhibition property in low concentration effectively; and in order to have desirable antimicrobial activity, they require higher concentration in the peripheral environments around the microbe. Then, the effective concentration about this microorganism was determined to be more than other microbes. According to all the previous researches on the consumed antibiotics with normal dose showed that the zone of growth inhibition of

these antibiotics using paper disc method Tetracycline, Penicillin, and Exaciline for *Staphylococcus aureus* were determined to be 20, 21, and 31 respectively (Ayaz *et. al.,* 2008; Tiwari *et al.,* 2004). Comparing to the results of the present research, it was specified that tea extract regarding inhibition effect was more effective and or is located at the same level of other common antibiotics. Since in order to prevent creation of antibiotic resistance in human body, consumption of antibiotics has pharmaceutical application in food and husbandry products is not authorized, and on the other hand, tea leaf extract has a high antioxidant property compared to other natural and artificial antioxidants (Mir Ahmadi, 1999). Then, it is suggested to use the above-mentioned extract as food concentrates applied in livestock section; and also in order to complete the study, synergistic or antagonistic property of tea leaf extract along with other antibiotics should be investigated.

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