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Improved Antimicrobial Efficiency of Aqueous Crude Extracts of Green Tea against Oral Microbiota

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ABSTRACT

As most of the plants have biologically active constituents, our present study focuses on the antimicrobial activity of aqueous crude extracts of green tea and black tea under aerobic and anaerobic incubation conditions using old extractive technique, maceration method by using both hot water and cold water and are subjected to evaporation to get semisolid consistency mass which are then air dried to get the powdery mass. Thus, obtained powders are stored in well closed containers and are made into solutions of desired concentrations of 25, 50, 75 and 100mg/mL to observe their antimicrobial activities against oral microbes under both aerobic and anaerobic incubation conditions thus inferring their antimicrobial activity against both aerobic and anaerobic oral microbes. The antimicrobial activity is determined by zones of inhibition shown by the extract dilutions which is compared to the zones of inhibition shown by commercial antibiotics used in oral cavity like tetracycline, gentamicin and amoxicillin. The cold water crude extract of green tea is found to have significant antimicrobial activity. The most prominent zone of inhibition is shown by the crude extract (cold water) combination of green tea and black tea in the ration of 1:1 at concentration of 75mg/mL and the zone of inhibition is found to be 31mm. Thus, inferring the extracts have significant antimicrobial activity against oral microbes and hence this investigation reveals the scope of formulating and developing dosage forms that can be used to treat oral infections.

Key words Crude extract, Green tea, black tea, Oral microbes, Zone of inhibition, Extract combination

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INTRODUCTION

The present study involves the use of plant extracts in the determination of their antimicrobial activity. As most of the plants are composed of biologically active chemical components and also several drugs in the present days are the analogues of active principles of plant origin.

In fact, scientific evidence supports that a good number of plant extracts have shown good inhibitory effect against pathogenic bacteria thus, supporting the development of antimicrobial agents. The present study aims to observe the antimicrobial activity of tea. Tea is one among many plant products that is used daily by most of the population across the countries. In the current study, the aqueous extract of green tea and black tea is investigated for their antimicrobial activity against oral microbiota which includes aerobic and anaerobic bacterial species [1]. The primary objective of using plant-based drugs is to overcome the unwanted effects of synthetic derivatives and thus enhancing the therapeutic efficacy and safer treatment [2]. In the present investigation, the aqueous crude extracts of green tea and black tea are checked for their antimicrobial properties. Green tea extract from the leaves of Camellia sinensis (green tea) is proven to have good range of antimicrobial activity due to presence of high content catechin, epigallocatechin gallate, EGCG [3-5]. In the present study the individual antimicrobial properties of aqueous crude extracts of green tea and black tea are assessed and the synergistic antimicrobial effect of green tea crude extract along with black tea is studied on both aerobic and anaerobic oral bacteria. The idea of using aqueous crude extracts is to support minimalistic chemical process and thus making the study cost effective.

MATERIALS AND METHODS

Materials

Samples of green tea and black tea used in the experiments are collected from region of Iraq. Thus procured samples of green tea and black tea, are processed to get aqueous crude extracts. The nutrient agar

medium to grow oral bacteria is procured from Neogen's Lab M, Lancashire, England.

Extraction methods

The crude extract of green tea and black tea is collected by maceration extractive technique. Maceration involves soaking of plant materials (coarse or powdered) in a stoppered container/flask with a solvent of choice and then allowed to stand at room temperature for a period of minimum 48- 72 hours with frequent agitation. This extraction method involves softening and breaking down of the plant's cell wall to release the soluble phytochemicals. After definite time interval , the mixture is pressed and filtered to get the extract. This filtrate is heated till a semisolid mass consistency is obtained. In this study, 10g of tea sample in 100mL of distilled water is considered.

Hot water extraction: In hot water extraction method, the green tea and black tea samples are macerated with hot distilled water of temperature 60^{O}C and left at room temperature for a period of 72 hours with occasional agitation. After time interval of 72 hours , the mixture is pressed and filtered through sterile filter paper (Whatman No.1 filter paper) to get the filtrate. This filtrate is evaporated over water bath till a semisolid mass consistency is obtained.

Cold water extraction: In cold water extraction method, the green tea and black tea samples are macerated with distilled water of temperature 25^{O}C and left at room temperature for a period of 72 hours with occasional stirring. After time interval of 72 hours , the mixture is pressed and filtered through sterile filter paper (Whatman No.1 filter paper) to get the filtrate. This filtrate is evaporated over water bath till a semisolid mass consistency is obtained.

Thus produced semisolid products by hot water extraction and cold water extraction are then air dried at room temperature, 27^{O}C till a powdery mass is obtained. Thus obtained extracts are stored separately in well closed sterile containers at 4^{O}C for further study. At the end of the extraction process we get the hot water extracts of green tea and black tea and cold water extracts of green tea and black tea.

Preparation of extract dilutions

The extract dilutions are prepared from the powdered extracts by adding required quantities of sterile distilled water to get the concentrations of 25, 50, 75 and 100mg/mL each. These dilutions are further used in the study.

Antimicrobial activity of aqueous crude extracts of green tea and black tea

Microbial culture preparation

Antimicrobial activity of aqueous crude extracts of green tea and black tea is evaluated by determining the zone of inhibition (mm). The efficacy is compared to potent antibiotics like gentamicin, tetracycline and amoxicillin which are widely used for the treatment of patients with wide range of oral infections caused by gram positive and gram negative bacteria [6].

Culturing of aerobic microbes of oral cavity

Oral microbial sample is collected from a human volunteer by swab technique [7]. Thus collected oral sample is placed in nutrient broth which is incubated for 24 hours and then the sample is taken from the broth and diluted with saline solution in the dilution tube of densi check apparatus till a bacterial density of 50 is reached. Bacteria density refers to number of bacterial cells per mL of solution. Thus, obtained diluted microbial suspension is used for inoculation over prepared brain heart infusion agar petri plates by streaking method.

Culturing of anaerobic microbes of oral cavity

Oral microbial sample is collected from a human volunteer by swab technique. Thus collected oral sample is placed in nutrient broth and shaken well for uniform distribution of bacterial suspension and then specified amount of broth is introduced into the vial of blood culture media which is incubated for 24 hours and then the sample is taken from the vial and diluted with saline solution in the dilution tube of densi check apparatus till a bacterial density of 50 is reached. Thus, obtained diluted microbial suspension is used for inoculation over prepared brain heart infusion agar petri plates by streaking method [8].

Preparation of nutrient media and petriplates

In the present study, the nutrient medium used is brain heart infusion agar and the techniques used is well diffusion technique to assess antimicrobial activity of the samples. The petriplates are marked as per the number of extract dilutions under study and then the autoclaved brain heart infusion agar medium is introduced into petriplates in laminar air flow cabinet to ensure sterile conditions and then the petriplates are allowed to dry under UV light for 10min and kept under laminar airflow cabinet for an hour to ensure complete solidification of nutrient medium under sterile conditions. These petriplates are inoculated with the microbial sample under sterile conditions to avoid cross contamination and left for 10min after which wells are made in the solidified nutrient medium with sterile borer. Into these wells the extract dilutions are introduced. Petriplate No. 01 has four wells with extract dilutions of 25mg/mL, 50mg/mL, 75mg/mL and 100mg/mL of hot water extract of green tea. Similarly petriplate no. 2,3,4,5,6,7,8 is introduced with the same concentration range of extract dilutions of both green tea and black tea respectively for both aerobic and anaerobic microbial culturing conditions [9]. The details of the petriplates, extract dilutions and incubation conditions are given in the Table 1.

Table 1: Petriplates with different extract dilutions.

Petriplate No.	Extract concentration (25,50,75 and100mg/mL)	Tea variant	Incubation condition at 37oC
1	Hot water extract	Green tea	Aerobic
2	Cold water extract		
3	Hot water extract	Black tea	
4	Cold water extract		
5	Hot water extract	Green tea	Anaerobic
6	Cold water extract		
7	Hot water extract	Black tea	
8	Cold water extract		

Note: Each petriplate has four wells with respective extract dilution concentrations ranging from 25, 50, 75 and 100mg/mL of 50µL each.

Thus, prepared petriplates of petriplate no.1 to 4 are incubated under aerobic conditions and petriplates of petriplate no.5 to 8 are incubated under anaerobic conditions for 24 hours and then observed for zone of inhibition. In the present investigation, the synergistic antimicrobial activity of aqueous crude extracts of green tea and black tea is observed by considering the concentrations in the ratio of 1:1 of hot water extracts and cold water extracts separately at aerobic and anaerobic incubation conditions. The details of petriplates with the combination of extracts are given in the Table 2.

As control, antibiotics like amoxicillin, tetracycline and gentamicin are used and cultured at both aerobic and anaerobic conditions and observed for zone of inhibition (mm) [10-12].

Table 2: Petriplates with extract dilution combinations.

Petriplate No.	Green tea:Black tea (1:1) (25,50,75 and100mg/mL)	Incubation condition at 37oC
9	Hot water extract	Aerobic
10	Cold water extract	
11	Hot water extract	Anaerobic
12	Cold water extract	

RESULTS

Antimicrobial activity of green tea and black tea aqueous crude extracts are determined against oral microbes. In this study, reference antibiotics that are used to treat oral microbial infections, like gentamicin, tetracycline and amoxicillin are used for screening the antimicrobial activity of aqueous crude extracts of green tea and black tea.

Antimicrobial activity of aqueous (hot water) crude extract of green tea

Green tea crude extract (hot water) showed significant zones of inhibition for random oral microbes under both aerobic and anaerobic incubation conditions showing the green tea crude extract (hot water) has significant effect against aerobic and anaerobic oral microbes. The hot water crude extract of green tea in the concentrations of 25, 50, 75 and 100 mg/mL has shown zones of inhibition (mm) of 25, 20, 27 and 27 mm respectively under aerobic incubation conditions.

Similarly, the hot water crude extract in the concentrations of 25, 50, 75 and 100 mg/mL has shown zones of inhibition (mm) of 22, 22, 21 and 28 mm respectively under anaerobic incubation conditions. The antimicrobial activity against oral microbes of crude extracts of green tea (hot water) under both aerobic and anaerobic incubation conditions is shown in the Table 3.

Table 3: Antimicrobial activity of aqueous (hot water) crude extract of green tea under aerobic and anaerobic incubation conditions.

S.No	Green tea aqueous extract concentration (mg/mL)	Zone of inhibition (mm)	
		Hot water	
		Aerobic	Anaerobic
1	25	25	22
2	50	20	22
3	75	27	21
4	100	27	28

Antimicrobial activity of aqueous (cold water) crude extract of green tea

The cold water crude extracts of green tea also showed significant zones of inhibition (mm) under both aerobic and anaerobic incubation conditions. The zones of inhibition of cold water crude extracts of green tea are given in the Table 4. The cold water crude extract of green tea has shown significant zone of inhibition at the concentration of 100mg/mL under aerobic incubation conditions.

Table 4: Antimicrobial activity of aqueous (cold water) crude extract of green tea under aerobic and anaerobic incubation conditions.

S.No	Green tea aqueous extract concentration (mg/mL)	Zone of inhibition (mm)	
		Cold water	
		Aerobic	Anaerobic
1	25	22	26
2	50	25	26
3	75	26	23
4	100	29.5	22

Antimicrobial activity of aqueous (hot water) crude extract of black tea

Black tea crude extract (hot water) has shown zones of inhibition for random oral microbes in both aerobic and anaerobic incubation conditions inferring the black tea crude extract (hot water) has effect against aerobic and anaerobic oral microbes. The hot water crude extract of black tea in the concentrations of 25, 50, 75 and 100 mg/mL has shown zones of inhibition (mm) of 14, 13, 17 and 20 mm respectively under aerobic incubation conditions.

Similarly, the hot water crude extract in the concentrations of 25, 50, 75 and 100 mg/mL has shown zones of inhibition (mm) of 21, 21, 23 and 22 mm respectively under anaerobic incubation conditions.

The antimicrobial activity against oral microbes of crude extracts of black tea (hot water) under both aerobic and anaerobic incubation conditions is shown in the Table 5.

The hot water crude extract of black tea has shown significant zone of inhibition at the concentration of 100mg/mL under anaerobic incubation conditions.

Table 5: Antimicrobial activity of aqueous (hot water) crude extract of black tea under aerobic and anaerobic incubation conditions.

S.No	Black tea aqueous extract concentration (mg/mL)	Zone of inhibition (mm)	
		Hot water	
		Aerobic	Anaerobic
1	25	14	21
2	50	13	21
3	75	17	23
4	100	20	22

Antimicrobial activity of aqueous (cold water) crude extract of black tea

The cold water crude extracts of black tea also showed significant zones of inhibition (mm) under both aerobic and anaerobic incubation conditions. When compared to hot water crude extract, the cold water crude extract of black tea has shown significant inhibitory effect at concentration of 100mg/mL under aerobic incubation conditions. The zones of inhibition of cold water crude extracts of black tea are given in the Table 6. The cold water crude extracts of black tea has shown significant inhibitory effect under aerobic incubation conditions.

Table 6: Antimicrobial activity of aqueous (cold water) crude extract of black tea under aerobic and anaerobic incubation conditions.

S.No	Black tea aqueous extract concentration (mg/mL)	Zone of inhibition (mm)	
		Cold water	
		Aerobic	Anaerobic
1	25	17	20
2	50	22	22
3	75	21	22
4	100	23	21

Synergistic antimicrobial activity of aqueous crude extracts of green tea and black tea in the ratio 1:1

In the present study, antimicrobial effect of aqueous crude extracts of green tea and black tea is also assessed in the ratio of 1:1 at varying concentrations of 25, 50, 75 and 100 mg/mL under both aerobic and anaerobic conditions.

It is prominent that the antimicrobial effect has enhanced when the aqueous crude extracts of green tea and black tea are combined. There is more significant synergistic antimicrobial effect in the concentration of 75mg/ml of cold water crude extracts of green tea and black tea (1:1) under aerobic incubation condition. The synergistic antimicrobial activity of aqueous crude extracts of green tea and black tea is given in the Table 7.

Table 7: Synergistic antimicrobial activity of aqueous crude extracts of green tea and black tea in the ratio 1:1.

S.No	Aqueous Extract Concentration (mg/mL) [Green tea:black tea=1:1]	Zone of inhibition (mm)			
		Aerobic condition		Anaerobic condition	
		Hot	Cold	Hot	Cold
1	25	24	22	23	25
2	50	24	25	22	26
3	75	26	31	24	23
4	100	27	29	20	25

In the present study, a comparison is made between the aqueous extracts of green tea and black tea and widely used antibiotics like gentamicin, tetracycline and amoxicillin which are used to treat oral infections. The antimicrobial activity of the above antibiotics is observed as control under both aerobic and anaerobic incubation conditions and is given in the Table 8. The standard antibiotic discs of standard concentration are used to assess the antimicrobial activity. The antimicrobial activity of the aqueous crude extracts of green tea and black tea is compared with the antimicrobial activity of standard antibiotics. Among the various concentrations of aqueous crude extracts, the concentrations at 75 and 100mg/mL have shown significant zones of inhibition.

Table 8: Antimicrobial activity of commercial antibiotics against oral microbes under aerobic and anaerobic conditions.

Antibiotic	Concentration (mcg)	Zone of inhibition (mm)	
		Aerobic condition	Anaerobic condition
Tetracyclin	30	7	6
Amoxicillin	20	9	11
Gentamycin	10	6	3

DISCUSSION

The hot water crude extract of green tea has shown significant zone of inhibition against aerobic and anaerobic oral microbes at the concentration of 100mg/mL under anaerobic incubation conditions. When compared to hot water crude extract, the cold water crude extract of green tea has shown significant inhibitory effect at concentration of 100mg/mL under aerobic incubation conditions. The antimicrobial activity against oral microbes of crude extracts of black tea (hot water) under both aerobic and anaerobic incubation conditions that is stated in table no.05 suggests the antimicrobial activity of aqueous (hot water) crude extract of black tea under aerobic and anaerobic incubation conditions is seen at 100mg/mL. Whereas the aqueous (cold water) crude extract of black tea has shown significant antimicrobial activity under aerobic

conditions. By considering the antimicrobial activities of green tea and black tea crude extracts, the activity of extract combinations of both green tea and black tea is studied under aerobic and anaerobic conditions. The study shows that the antimicrobial activity is enhanced and most significant antimicrobial activity is observed when the cold water extracts of green tea and black tea are combined at comparatively lesser strength of 75mg/mL in the ratio of 1:1 under aerobic conditions. This investigation suggests to use the cold water to get the aqueous crude extracts of green tea and black tea to reap the maximum antimicrobial benefits and thus, the combination of aqueous crude extracts of green tea and black tea in equal proportions has shown significant antimicrobial activity under aerobic conditions. This study can be further carried out to formulate suitable oral hygienic products.

CONCLUSION

In the present investigation the antimicrobial activity of widely used plant product in our daily use is assessed in various concentrations of its aqueous crude extract. The crude aqueous extract is preferred to avoid use of various organic solvents and to keep the cost of observational study minimal. The extracts are obtained by using hot water and cold water to observe the effect of temperature on active principles of plant product. In this observational study, the cold water crude extracts have shown significant antimicrobial effect when compared to hot water crude extracts. From the observations, it is evident that the extracts used in this study has significant antimicrobial activity against oral microbes and thus paving ways for further research on oral antimicrobial activity. The optimized concentration is selected based on the zone of inhibition and can be further processed into suitable formulations that can be used in oral cavity thus showing that the present investigation has significant scope of wide variety of further research in developing pharmaceutical dosage forms that are effective against oral infections.

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