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Investigation of Biochemical Usefulness of Tea (Camellia sinensis) Flower

Camellia sinensis Çay Çiçeğinin Biyokimyasal Yararlılığının Araştırılması

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Abstract

Tea is one of the most preferred beverages in the world, following water. The content of the tea consists of bioactive polyphenols, especially catechins, which play acrucial role in the quality of tea and human health.

In this study, total phenolic content, DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity and total flovonoid content were analyzed to determine the antioxidant properties of *Camellia sinensis* tea flower. To determine the general chemical characteristics and antimicrobial properties of tea flower; phenolic content and sugar content were analyzed by high performance liquid chromatography (HPLC) and antimicrobial activity was evaluated by disc diffusion technique.

As a result of the analysis tea flower, showed prominent results in terms of the amount of phenolic compounds and antioxidant activity. The main phenolic compound identified from the methanolic extract of the tea flower was determined as catechin with a value of 128.126 mg / kg.As a result of the sugar content analysis by HPLC; only Fructose, Glucose and Sucrose were determined among the ten sugar standards used. Besides, it was concluded that when samples were heated, sugar contentsvalue were increased almost double.In the study, 5-gram negative, 3-gram positive and 2 fungi species were used for antimicrobial analysis. As a result of antimicrobial analysis performed

Özet

Çay, suyun ardından dünyada en çok tercih edilen içeceklerden biridir. Çayın içeriği, başta kateşinler olmak üzere çayın kalitesinde ve insane sağlığında çok önemli rol oynayan, biyoaktif polifenollerden oluşmaktadır.

Bu çalışmada, Camellia sinensis çayçiçeğinin antioksidan özelliklerini belirlemek için, toplam fenolik içerik, DPPH (2,2-difenil-1pikrilhidrazil) radikal temizleme aktivitesi ve toplam flovonoid içeriği analiz edilmiştir. Çay çiçeğinin genel kimyasal karakteristiğini ve antimikrobiyal özelliğini belirlemek için; yüksek performanslı sıvı kromatografi (HPLC) ile fenolik içeriği ve şeker içeriği analiz edilirken, disk difüzyon tekniğiyle de antimikrobiyal aktivitesi değerlendirildi.

Analiz sonucunda çay çiçeği, fenolik bileşiklerin miktarı ve antioksidan aktivite açısından belirgin sonuçlar gösterdi. Çay çiçeğinin metanolik ekstraktından tanımlanan ana fenolik bileşik, 128.126 mg / kg değeriyle kateşin olarak tespit edilmiştir. HPLC ile yapılan şeker içeriği analizi sonucunda, kullanılan on standarttan sadece Fruktoz, Glukoz ve Sukroz tespit edilmiştir. Bunun yanında örnekler ısıtıldığında şeker içeriğinin neredeyse iki katına çıktığı bulunmuştur

Çalışmada antimikrobiyal analiz için 5-gram negatif, 3-gram pozitifve 2 mantar türü kullanıldı. Agar disk difüzyon tekniğiyle yapılan antimikrobiyal analiz sonucunda kullanılan on suşun hepsine karşı çay çiçeğinin

by agar disc diffusion technique, tea flower was found to be sensitive to all ten species used.

According to the results of this preliminary analysis study; *Camellia sinensis* tea flower could be preferred as much as tea leave and future studies should be focused on similar researches.

Keywords: Tea flower, *Camellia sinensis*, Antioxidant activity, HPLC, Phenolic content, Antimicrobial activity, Sugar content.

duyarlı olduğu bulundu. Elde ettiğimiz bu ön çalışma sonuçlarına göre, Camellia sinensis çay çiçeğinin çay yaprağı kadar tercih edilebileceğini ve gelecekteki çalışmaların benzer araştırmalara odaklanması gerektiğini göstermiştir.

Anahtar kelimeler: Çay çiçeği, *Camellia sinensis*, Antioksidan aktivite, HPLC, Fenolik içerik, Antimikrobiyal aktivite, Şeker içeriği.

Abbreviations: HPLC, High Performance Liquid Chromatography

1. INTRODUCTION

Obtained from the leaves of the *Camellia sinensis (C.sinensis)* plant, tea is one of the most preferred beverages in the world, following water. Tea production and cultivation is carried out in in more than thirty countries. Tea is brewed by different methods in many of the cultures and countries. In different geographical regions of different countries, many herbs are brewed and consumed as a tea. Annual per capita consumption of tea are the top five countries are that Ireland (3.17kg), Kuwait (2.66kg), the UK (2.46kg), Turkey (2.36kg), Qatar (2.00kg).

Turkey, in terms of the area under cultivation of tea in the world 6th, 5th in terms of dry tea production, and 4th in terms of annual per capita consumption ranks. *C. sinensis* is harvested in 3 or 4 times a year, especially in May, July and September depending on the annual weather conditions, in the Province of Rize, Eastern Anatolia in Turkey.

It was produced a total of 655.285 tons of tea in Turkey in 2012 and 446.377 tons produced only in Rize (Statistical Bulletin, 2012). That means nearly %70 of total product generated in Turkey.

Tea from *C. sinensis* is processed by different manufacturing processes. Consequently, they are named according to the production method used. White and green tea have no fermentation, Oolong tea is semi-fermented and black tea is fully fermented (Santana-Rios, Orner, Amantana, Provost, Wu and Dashwood, 2001a). There is also another type of tea called pu-erh tea. The puerh tea processing involves microbial fermentation by *Aspergillus niger*, which is different from the processing of the other types of tea (Pinto, 2013). Different manufacturing processes also affect the characteristic color and flavor of tea.

It was reported that plants and fruits contain a highly biologically active compound that protects them from a variety of physical and chemical hazards, such as diseases, parasites, bacteria (Kolayli, Kara, Tezcan, Erim, Sahin, Ulusoy and Aliyazicioglu, 2010).Tea has a lot of co-extracts such as caffeine, polyphenols, sugars, organic acids and pigments, and high levels of chlorophyll in particular. The percentage of these substances strongly depend on the type of tea; and black tea is considered to be the most difficult matrix due to rich interfering substances (Sadowska-Rociek, Surma and Cieślik, 2014). Tea contains beneficial, bioactive polyphenols, mostly the catechins, which play a crucial role in tea quality and human health. Many studies showed that polyphenols present in tea decreases the risk of developing a disease mostly (Mukhtar and Ahmad, 2000). The health benefits of tea stem from the antioxidant character of tea polyphenols; in addition to other important biological mechanisms. From a biological point of view, tea is a mixture of numerous bioactive compounds, including catechins, flavonols, lignans, and phenolic acids. Tea leaves include epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate, catechins, theaflavins and thearubigins, and flavonols, such as quercetin and myricetin, as well as the nitrogenous compounds such as caffeine and theobromine (Yuan, 2013; Lambert and Yang, 2003). Although tea polyphenols are antioxidant, they may also produce reactive oxygen species (ROS). Epigallocatechin-3-gallate, the main polyphenol content of green tea, binds to several receptors and signaling

molecules, in addition to inhibitory function of main receptors, kinases, proteinases and other enzymes (Yang, Wang, Lu and Picinich, 2009a). Tea polyphenols are also strong chelators of metal ions; the formation of ROS due to auto-oxidation of many compounds is prevented by the chelation of free metal ions (Yang et al., 2009a). Based on these results, we suggest that tea, being readily available and widely consumed, has a high potential use in the prevention of human cancer. However, the cancer-preventive activity of tea has not yet been observed consistently in studies in humans (Yang et al., 2009a). Green tea consumption is one of the means of flavonoid intake. Green tea has been shown to possess cancer-preventing activities in many cancer types (Lee, Bode and Dong, 2011).

The flavonoids content in flowers of tea has been reported to be higher than the leaves (Yang et al., 2007). Tea flower also showed strong antioxidanteffects (Chen, Li, He, Li, Tsoi, Zhai and Kurihara, 2012). Unfortunately, less attention has been given to flower of tea (*Camellia sinensis*). In fact, tea flower, as well as tea, has been considereda healthful beverage since ancient times, especiallyin southern China (Chen et al, 2012).

Compared with tea leaves, tea flowers have similar chemical compositions (Yung et al., 2003). Tea flowers contained many nutrition compounds, such as protein, sugar, sucrose, vitamin, amino acid, tea polyphenols and caffeine (Yang et al., 2007). From this aspect, it can be concluded that tea flowers also have important application value as leaves.

2. MATERIALS AND METHODS

The methanolic extract from the flower of *C. sinensis* was obtained from cultivated tea in Rize,Pazar in Turkey.

2.1. Preparation of Methanolic Extract

Methanolic extracts of the *C. Sinensis* flower was used for antioxidant and phenolic analyses. Ten to fifteen-gram samples of tea was placed in a falcon tube (50 mL) and 50 mL 99% methanol was added. The mixture was continuously stirred with a shaker (HeidolphPromax 2020, Schwabach, Germany) at room temperature for 24 h. Particles were removed with Whatman's filter paper. The final volume of the solution was adjusted with methanol. The methanolic extract was divided into two parts, the first being used for antioxidant tests and the second for phenolic analysis.

2.2. Determination of Total Phenols

The total phenolic content was determined using the Folin−Ciocalteu assay (FC-GAE) (Slinkard& Singleton (1977), and the antioxidant capacity was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH). All of these well-established assays (DPPH, Folin−Ciocalteu, Total Phenolic Content) have been used for determining the antioxidant capacity of tea flower.

Total phenol content (TPC) of tea flower was determined spectrophotometrically in accordance with the modified method of Slinkard and Singleton, 1977 through Folin− Ciocalteu's reagent. First, 20 µL of the sample was added into test tube containing 400µL 0.5 N of Folin−Ciocalteu's reagent, 680 µL of distilled water, and 400 µL of 10% Na_2CO_3 and vortexed afterwards. After 2 h, the absorbance of blue coloration was measured at 760 nm against a blank sample. Gallic acid was used as the standard, and the results were expressed as milligrams per liter of gallic acid equivalents (GAE). The polyphenol concentration in the samples was derived from a standard curve of absorbance of gallic acid concentrations, ranging from 0.03125 to 1 mg/mL. All measurements were performed in triplicate.

2.3. Determination of Free Radical Scavenging Ability by the DPPH Method

DPPH • radical (2,2-diphenyl-1-picrylhydrazyl) can be purchased commercially. In order to obtain a 100 µm solution, a stable radical was dissolved in 100 mL of methanol. A 750 µL of the extract was added to 750 µL of 100 µM DPPH in methanol. The free radical scavenging capacity of the sample was assessed by measuring the absorbance at 517 nm after 50 min. DPPH • solution and solvent sample were used as the blind. And Trolox (0.000625 to 0.02 mg/mL) was used as the positive control. Values obtained were plotted against concentration (mg/mL) of sample dilutions, and the final results are expressed as IC_{50} values (concentration of samples required for scavenging 50% of the

DPPH radicals). The antioxidant capacity was expressed as SC_{50} mg/ml Trolox equivalents, by making use of the calibration curve of Trolox. All determinations were performed in triplicate (Potterat, 1997).

2.4. Determination of Total Flavonoid Content

The amount of total flavonoid was measured with a spectrophotometric method as reported previously (Fukumoto &Mazza, 2000) using quercetin as standard. Briefly, 0.5 mL methanolic samples, 0.1 mL of 10% Al(NO3)3 and 0.1 mL of 1 M NH4.CH3COO were added to a test tube. After 40-min incubation at room temperature, the absorbance was measured against a blank at 415 nm. The standard calibration curve was plotted using quercetin. The total flavonoid concentration was expressed as mg of quercetin equivalents per 100 g sample (mg QE/g).

2.5. Determination of Phenolic Content by the HPLC Method

First, dried white flower of *C.sinensis* (5g) was extracted by 100mL methanol at room temperature, standing for 24 h. The extract was then filtered and the filtrate was allowed to evaporate in a rotary evaporator. Then Diethly ether Chloroform (10mLx2) was added to the concentrated solution in order to remove the phenolic content. The aqueous phase was collected and extracted with ethyl acetate (10mLx2); subsequently the tea extracts were combined. The tea extracts and individual phenolics were prepared as the stock solutions, for HPLC and spectrophotometric measurements. Working (standard) solutions with different concentrations were prepared by dilution and filtered (0.45 mm, Millipore) before HPLC injection.

2.6. HPLC Method for Phenolics

Twenty of phenolic compounds were analyzed using HPLC (Thermo Finnigan Surveyor), in a UV-Vis detector supplying a double wavelength simultaneously. Two different wavelengths, 280 and 315 nm, were used to detect phenolic substances. The sample was injected into the HPLC system with a reverse phase C18 column (150 mm x4.6 mm, 5 µ; Fortis). Acetonitrile, water and acetic acid were used as mobile phase by applying the programmed gradient (De Villers et al., 2004). The mobile phase consisted of (A) 2% acetic acid in water and (B) acetonitrile:water (70:30). Injection of sample volume was 25 µL, column temperature was 30 °C and flow rate was 1.2 mL/min. The solvent programmed began with a linear gradient used was held at 95% A for 3 min, decreasing to 80% A at 10 min, 60% A at 20 min, 20% A at 30 min and and finally 95% A at 50 min (Akyuz, Şahin, Islamoglu, Kolayli and Sandra (2014).

An Agilent 1100 liquid chromatography system, equipped with a Hypersil ODS C18 column (4.0--150 mm), was used for the analyses. All determinations were performed at 25 °C. Mobile phases had an aqueous solution of 2% A Acetic acid- ultra water and 70-30% B Asetonitril-ultra water.

2 % acetonitrile (A) and methanol (B). Gradients of 0-50% B for 0-12 min and 50-100% B for 13-20 min were used. The mobile phases were filtered (0.45 mm, Millipore) and degassed prior to use, and a flow rate of 1.2mL/min-1 was employed. The sample injection volume was 20 µl, and the detection was performed at 280 nm. Analytes' chromatographic peaks were identified by comparing the retention times with those of the standard. Quantification was made by peak integration using external standards. The summed individual catechins were used to determine the total catechins of the tea extracts.

2.7. Determination of Sugar Content by the HPLC Method

First, dried white flower of *Camellia sinensis* (2 g) flower was added by 20 mL water at room temperature, standing for 5 min. shaking and separated two parts. One part of sample extract was directly filtered (A) and used for HPLC. The other part of sample was boiling at 5 min (B) and after filtered, used for HPLC. The tea extracts and individual references were prepared as the stock solutions, for HPLC.

2.8. HPLC Method for Sugars

The composition of water-soluble sugar composition analysis was performed by high-performance liquid chromatography (HPLC,Elite LaChrom, Hitachı HPLC) with a reverse phase -

NH_2column (200/ 4,6 Nucleosil 100-5 NH_2).RI detector was used for determination of tea flower sugar composition. Mobile phase flow rate 1,5 mL/min, temperature 45^oC, the sample injection volume was 20 µL. 79% acetonitrile, 21% pure water for 15min were used in isocratic mode (Bogdanov, Martin and Lullmann, 2002).

The tea extracts and sugars were prepared as the stock solutions for HPLC. Working (standard) solutions with different concentrations were prepared by dilution and filtered (0.45 mm, Millipore) before HPLC injection. The following sugars were used as references: Arabinose, Xylose, Fructose, Glucose, Galactose, Sucrose, Maltose, Theralose, Melebiose, Melezitose. Quantification was made by peak integration using external standards.

2.9. Antimicrobial Activity

Escherichia coli ATCC®25922, *Bacillus subtilis* ATCC®6633, *Proteus vulgaris*ATTC®13315, *Micrococcus luteus* ATTC®9341, *Staphylococcus aureus* ATTC®25923, *Pseudomonas aeroginosa* ATTC®10145, *Saccaromyces cerevisiae* ATTC®9763, *Klebsiella pneumoniae* ATTC®13883, *Aspergillus niger* ATTC®9642, *Yersiniaent erocolitica* ATTC®27729 strains were used for antimicrobialactivity. These strains were obtained from ATCC (American Type Culture Collection).

The gram-positive bacteria studied were *Bacillus subtilis* ATCC®6633, *Staphylococcus aureus* ATTC®25923, *Micrococcus luteus* ATTC®9341; The gram-negative bacteria studied were *Klebsiella pneumoniae* ATTC®13883, *Yersinia enterocolitica* ATTC®27729, *Pseudomonas aeroginosa* ATTC®10145, *Proteus vulgaris* ATTC®13315, *Escherichia coli* ATCC®25922.

Antimicrobial activity of methanolic extract of *C. sinensis* flower with standart antibiotics was assessed against 5 gram-negative bacteria, 3 gram-positive bacteria and 2 fungi by using the agar disc diffusion tecnic (Chanda et al., 2011; Sokmen, Jones and Erturk, 1999).

The petri dishes were prepared by pouring 20 mL of sterilized Mueller Hinton agar for bacteria and Sabouraud dextrose agar for fungal strains, which was then seeded with 200 µL of test culture containing 1×10^8cfu/mL for bacteria and 1×10^7spores/mL for fungal strains. The media were allowed to solidify. All bacteria plates (6 mm) incubated for 24 hours at 37 °C and fungal strains 48 hours at 27 °C. Results (Table 4) were recorded by measuring the zone of inhibition appearing around the disks.

3. RESULTS AND DISCUSSION

There is a limited number of studies carried out on tea flowers, despite the numerous studies on tea leaves in the literature. Although health benefits of tea leaves have widely been studied, lesser attention was given to the flowers of the tea plant. Recent studies found that tea flowers contain numerous bio-active compounds, suggesting that tea flowers can be a potential raw material in industries in areas such as healthcare, food, pharmaceutical, flavor, and cosmetic (Chen, Mei,Jin,Kim,Yang and Tu (2014). Biological, anti-oxidant, gastroprotective, hypoglycemic, antihyperlipidemic, antiallergic, anti-proliferative and apoptotic effects may be due to the fact that tea flowers include similar chemical compounds as tea leaves (Joshi & Gulati, 2011; Lin, Wu and Lin(2003); Yang et al., 2007, 2009b,Way et al.,2009, Yoshikawa, , Morikawa, Yamamoto, Kato, Nagatomo and Matsuda (2005);Yoshikawa,Nakamura, Kato, Matsuhira and Matsuda (2007). Thus, tea flowers may also have important uses as tea leaves.

There are various studies conducted on the antioxidant properties of certain teas (Yıldırım, Mavi, Oktay, Kara, Algur and Bilaloğlu, 2000); Joshi et al.2011, Dalar & Konczak, 2013). It has been reported that there is an inverse relationship between dietary intake of antioxidant-rich foods and incidence of a number of human diseases (Rice-Evans, Sampson, Bramley and Holloway, 1997). In addition, antioxidant compounds, which are responsible for these properties, could be isolated and then used as food additives to delay spoilage of foods due to oxidation (Yıldırım et al., 2000). Hence, researches on identifying antioxidant-rich foods are important. Other important compounds found in tea are the polyphenols. Polyphenols in green tea can protect against 1 mM H_2O_2 induced damage in bladder cells, with epicatechingallate (ECG) displaying high protective properties against three bladder cell lines (Coyle, Philips, Morrisroe, Chancellor and Yoshimura(2008). It was suggested that the observed anti-IL-8 and antioxidant activity may

be stemming from the polyphenolic components in tea extracts (Thring, Hili and Naughton, 2011).

As another benefit, the tea polyphenols have been proposed to counteract the decrease in metabolic rate, observed usually during weight loss (Hursel & Westerterp, 2013).

It is thought that daily consumption of tea (*C. sinensis*) has benefits on health, such as prevention of lung, skin, bladder, esophageal, and gastrointestinal cancers (Lambert and Yang, 2003; Lu, Liao, Yang, Reuhl, Hao and Yang (2006); Santana-Rios, Orner, Xu, Izquierdo-Pulido and Dashwood, 2001b).

In our study, the total phenolic compounds, determined by the FolinCiocalteu method, are reported as gallic acid equivalents by reference to a standard curve (y = 1.274x + 0.027, R^2 = 0.999). Total flavonoid contents were (16.393 ± 0.033) mg quercetin equivalent (QE) /g of extract, by reference to a standard curve (y = 6.703x + 0.010, R^2 = 0.999(Table. 1).

Table 1. Antioxidant activities and total phenolic contents in methanolic extract of tea flowers (*C. sinensis*)

Parameter	Sample of tea flower
Total phenolic content (mg GAE/g)	28.641±0.742
Total flavonoids content (mg Quercetin/g)	16.393±0.033
DPPH Radical Radical Scavenging Activity (Trolox mg/mL)	0.0843± 0.001

Tea flowers yielded mean DPPH IC_{50} values of 84.25 µg/ml. Trolox is used as a positive control for the scavenging activity with a mean IC_{50} value of 69.03 µg/ml (R^2 =0. 996).The lower IC_{50} value implies the higher antioxidant activity. Antioxidant activities, total phenolics (24.591±0.091) and flavonoids (8.795±0.540) of parts of Anzer tea were correlate the our results Turumtay, İslamoğlu, Çavuş, Şahin, Turumtay andVanholme (2014).

In this research study, 20 different constituents such as gallic acid, protocatechuic acid, *p*-OH benzoic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid,syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, rutin, myricetin, fisetin, quercetin, apigenin, kaempferol, isorhamnetin, rhamnetin were investigated. Phenolic compounds, such as gallic acid, protocatechuic acid, *p*-OH benzoic acid, catechin, *p*-coumaric acid, myricetin, apigenin were all quantified by HPLC-DAD in the methanol extracts of the tea flower (Table. 2).

Table 2. Phenolics in methanolic extract of tea flowers (*C. sinensis*)

Standard	Tea flowers (mg/ kg)
gallic acid	112.714
protocatechuic acid	4.902
p-OH benzoic acid	13.728
catechin	128.126
p-coumaric acid	9.216
myricetin	17.614
apigenin	5.108
kaempferol	7.502
chlorogenic acid	ND[a]
vanillic acid	ND[a]
caffeic acid	ND[a]
syringic acid	ND[a]
epicatechin	ND[a]
isorhamnetin	ND[a]
ferulic acid	ND[a]
rutin	ND[a]
o-coumaric acid	ND[a]
fisetin	ND[a]
quercetin	ND[a]
isorhamnetin	ND[a]

[a]Non-detected.

As a result of the composition of water-soluble sugar analysis sugar contents mainly consist of Fructose, Glucose, Sucrose (Table 3). Arabinose,Xylose, Fructose, Glucose, Galactose, Sucrose, Maltose, Theralose, Melebiose, Melezitose used as sugar references materials.

Table 3. Sugar contents of tea flower (*C. sinensis*)

Standard	A	B
Ara.%	ND[a]	ND[a]
Malt.%	ND[a]	ND[a]
Melb.%	ND[a]	ND[a]
Ther.%	ND[a]	ND[a]
Gal.%	ND[a]	ND[a]
Xyl.%	ND[a]	ND[a]
Melez.%	ND[a]	ND[a]
Fruc.%	4,19±0,11	9,15±0,21
Glu.%	3,84±0,35	9,13±0,13
Suc.%	1,86±0,11	4,78±0,06

[a]Non-detected.Ara.(Arabinose), Xyl.(Xylose), Fruc.(Fructose), Glu.(Glucose), Gal.(Galactose), Suc.(Sucrose), Malt.(Maltose), Ther.(Theralose), Malb.(Melebiose), Melez.(Melezitose).

It was evident from the findings that the antioxidant and antimicrobial properties of these

plants were due to their phenolic contents. In this study also observed antimicrobial activity on bacteria and fungus. Comparing the eight bacteria studied, ıt is clear that M. l. *Micrococcus luteus* is most sensitive (Table 4). In literature after the *Bacillus cereus, Micrococcus luteus* was also found to be second most sensitive strain (Almajano, Carbo, Jiménez and Gordon (2008). It was expressed as the bactericidal activities of the teas could be accounted for by the levels of catechins (Friedman, Henika, Levin, Mandrell andKozukue, 2006). Our results also support these findings.

Table 4.Effect of tea (*C. sinensis*) flower methanolic extract on antimicrobial activity (diameter of the inhibition zone measured in mm)

Microorganisms	**Inhibition zone (mm)**
Escherichia coli ATCC®25922	12.5
Bacillus subtilis ATCC®6633	14.3
Proteus vulgaris TTC®13315	14.6
Micrococcus luteus TTC®9341	16.9
Staphylococcus aureus ATTC®25923	11.8
Pseudomonas aeroginosa ATTC®10145	15.3
Yersinia enterocolitica ATTC®27729	13.8
Klebsiella pneumoniae ATTC®13883	16.3
Aspergillus niger ATTC®9642	13.0
Saccaromyces cerevisiae ATTC®9763	14.3

4. CONCLUSIONS

According to the results stated above, it was concluded that the tea (*C. sinensis*) flower methanolic extract includescatechins are themost abundant as in the literature. Compared with tea leaves, tea flowers have similar chemical compositions and contain less caffeine but comparable amounts of total catechins. Tea flowers contained many nutrition compounds, such as protein, sugar, sucrose, vitamin, amino acid, tea polyphenols and caffeine (Yang et al., 2007). But generally the studies reported in the literature, which are usually about tea flower polysaccharides.

The present study demonstrated that tea flowers contain appreciable amounts of polyphenols and tea flower has potent antimicrobial activity on examined microorganisms. In the our present study, we have demonstrated that the tea flower methanolic extract had inhibitory effects on the DPPH radical. Our results also demonstrate that the phenolic catechin are the most rate in the tea flower. The outputs of this study offer researchers advance investigation on antimicrobial properties against firstly *Micrococcus luteus* and possibly other our studied microorganisms. The data also offer the consumer a choiceof tea brands with high antimicrobial properties against firstly *Micrococcus luteus* and the other previously mentionited microorganisms. But *Micrococcus luteusis* a probiotic gram positive bacterium that is also used in the prevention of bacterial diseasesand also *Saccaromyces cerevisiae* known as baker's yeast. They are not pathogenic microorganism. These results indicated that tea flowers might exhibit beneficial health properties and might be suitable for making an alternative to tea beverage. It is likely that tea flowers might be useful for making alternative tea beverages

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