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Research paper

The Potential of Antibacterial and Antioxidant Activities of Oolong Tea Residues

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Abstract

Traditionally, the residues of the Oolong tea were believed to help in reducing beriberi symptoms. However, these residues are often discarded without optimizing its potential. Thus, the aim of this study is to screen the potential of Oolong tea residues in terms of their antibacterial and antioxidants properties by using the microdilution method and DPPH (1,1-(diphenyl-2-picrylhydrazyl) Scavenging Activity method. In this study, the tea residues were tested up to five cycles of usage. The antibacterial properties of the residues were tested against several Gram positive and negative bacteria. Results showed the highest percentage of inhibition was observed in *S. faecalis* (50.8 ± 1.3%), *B. subtilis* (49.0 ± 2.3%), *S. typhimurium* (48.8 ± 1.5%), *S. aureus* (47.8 ± 2.1%), *E. coli* (43.5 ± 3.6%) and *P. vulgaris* (35.0 ± 4.0%). The first cycle of the tea residues showed highest percentage of inhibition in all bacteria tested but the antibacterial properties showed significant decrease as the cycle increases. For the antioxidant activities, the colour changed from purple to yellow after the fifth residue cycle suggests the presence of secondary compound such as flavonoid. The first cycle of the Oolong tea residues displayed 56.0% DPPH inhibition at 10 g/ml and 85.9% inhibition at 50 g/ml. Yet, the antioxidants activity of the Oolong tea residues decreased with the increase of the residue cycle. This indicates the effectiveness of Oolong tea residues decreases after several times of usage.

Keywords: *Oolong Tea; Antibacterial; Antioxidant; DPPH; Phytochemical Screening.*

1. Introduction

In Malaysia, bacterial contamination related with food poisoning cases tend to increase every year [1]. Even though lots of potential antibiotics have been developed, most of them are costly and some could cause allergy reactions [2]. In addition, human is constantly exposed to free radicals through external sources which include pollution, toxic metals, cigarette smoke and pesticides which are dangerous as it causes damage towards cell and tissues leading to age related disorders such as impairment and Parkinson's diseases [3]. Thus, a natural and traditional medicine seems to have a great potential to solve the problems. Plants contained bioactive molecules with therapeutic potential that serves as remedies in treating diseases [4]. Up to 61% of new drugs developed in 1981 – 2002 were based on natural products and have been successful in treating infectious disease [5]. This is due to plants are rich with secondary metabolites such as tannins, terpenoids, alkanoids, flavonoids and glycosides and these compounds were found to have antimicrobial properties [5]. Oolong tea (Camellia sinensis) is one of the most widely consumed beverages in the world for its specific aroma, taste and putative positive physiological functions [6]. It is a type of semi fermented tea that hold numerous compounds with health benefit properties such as polyphenols and vitamins [7]. In addition, Oolong tea is also known as an antioxidant, anti-hyperglycemic and anti-obesity agent [6, 8]. Moreover, tea is believed to eliminate toxins, improve blood flow and improve body resistance towards diseases as it is rich of flavonoids and other antioxidant polyphenols [6]. Commercialized Oolong tea is usually classified into three major categories; unfermented tea that contain catechins; semi-fermented black tea which contain both catechins and theaflavins and fully fermented black tea that contain catechins, theaflavins and polymeric thearubigins [6, 9]. Although they come from the same plant, they differ in terms of appearance, orgaholeptic taste, chemical contents as well as their flavour which is due to different fermentation process [10]. However, not much study was focus on the residue of Oolong tea which is often discarded after a single used without being maximized its potential. The Oolong tea residues are strongly believed to have a potential as natural source of antimicrobial agents [8]. Thus, the aim of this research is to seek the potential of antimicrobial and antioxidant activities of the Oolong tea residues. The finding of this research is important to maximize the use of the Oolong tea residues and discover its potential as therapeutic agent in the future.

2. Methodology

2.1. The preparation of Oolong tea residues

An amount of 50 g of Oolong tea was purchased from the local market and blended into fine powder. The powder was then soaked in 1 L of boiling distilled water for five minutes before the tea was filtered to remove the water [11]. The resulting residue is then la-belled as the first cycle of the Oolong tea residues and designated as A. To obtain the second cycle of the residues, the

same filtration process was repeated twice, and this process will also be repeated to obtain the third, fourth and fifth cycle of the tea residues which was designated as B, C, D, and E respectively. The pH of the Oolong tea residues at each cycle of the filtration was recorded by using a pH meter.

2.2. Bacterial cultures and maintenance

Laboratory cultures of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Proteus vulgaris*, and *Streptococcus faecalis* were used in this study. The bacteria cultures were obtained from the Biology Lab, UiTM Perlis. These bacteria were maintained in Nutrient Agar (NA) at -20°C and subculture when required.

2.3. Antimicrobial assay using broth micro dilution method

Each of the bacteria cultures were grown overnight in Nutrient Broth (NB) at 37 °C with 150 rpm. The following morning, the cultures were transferred to fresh NB at 1:100 dilution and grown for approximately three hours until it reached the exponential phase. The turbidity of the bacterial suspension was then adjusted to match with the 0.5 McFarland standard [12] which is equivalent to 10 X 10^8 cells/ml. Subsequently, 1 ml of this bacteria culture was added to 1 ml of the first cycle (A) of the Oolong tea extract giving a final volume of 5 X 10^7 cells/ml [13].
The same process was repeated for the 2nd, 3rd, 4th and 5th cycle of the Oolong tea residues. For the positive controls, 1 ml of the bacterial culture was added to ampicillin instead of the Oolong tea. For the negative controls, 1ml of the bacterial cultures was added to 1 ml of sterile distilled water which replaced the tea residues. All the tubes were incubated overnight at 37 °C.
Following incubation, the turbidity of the bacteria culture in each tube was recorded at absorbance of 600 nm. Hence, the percentage of growth inhibition in each of the tube can be determined using the following formula [14];

% of Inhibition= OD600 sample- OD600 negative control X 100%

2.4. DPPH scavenging activity

DPPH powder of 0.005 g was added into 12 mL of methanol in a conical flask. A volume of 1 mL of sample A, B, C, D and E was added to 1 mL of freshly DPPH solution in methanol. After 20 minutes of incubation at room temperature, the absorbance of the solution was measured at 517 nm using UV-Vis spectrophotometer. The DPPH assay was repeated with sample B, C, D and E to estimate the reducing agent in each of the sample related to antioxidant activity [15]. The data was illustrated the present of antioxidant activity.

% of Inhibition= OD of control- OD of test/ OD of control X 100

2.5. Statistical analyses

Single factor of ANOVA at 5 % level of significance ($P < 0.05$) was carried out to analyse the data points using SPSS statistical program version 20 to check the statistical differences between the groups of data with different combinations [16]. All values were expressed as the mean of 3 completely independent replicates, and standard deviations was evaluated for all mean values [17].

3. Results and discussion

3.1. pH value of Oolong tea

pH value plays important role in identifying the acidic or basic level of solution. Table 1 shows the pH value of Oolong tea from first to fifth cycles of usage.

Table 1: pH Value of Ascorbic Acid for First Time Use and Oolong Tea from First to Fifth Cycle Usage

Cycle Designation	pH
1 A	4.54
2 B	4.60
3 C	4.71
4 D	4.82
5 E	4.90

Based on Table 1, pH value was recorded around 4.54 to 4.90 from first to fifth cycles of the usage. This indicate, for every time usage, the solution became less acidic due to the extract had been diluted. Even though less acidic conditions will encourage the bacteria growths [18], there is no significant different in pH value from first to fifth cycle usage.

3.2. Percentage of inhibition of bacteria by micro dilution method

The ability of bacteria to inhibit Oolong tea residues after five cycles of usage was carry out by using infusion method. This is to mimic the consumption of tea by human. Table 2 shows the percentage of bacteria inhibition influenced by five cycles of Oolong tea usage.

Table 2: The Percentage of Bacterial Inhibition Influenced by Five Cycles of Oolong Tea Usage

% inhibition	Gram positive			Gram negative		
Samples	*B. subtilis*	*S. faecalis*	*S. aureus*	*P. vulgaris*	*S. typhimurium*	*E. coli*
A	48.97 ± 2.345	50.76 ± 1.33	47.83 ± 2.08	35.01 ± 4.04	48.77 ± 1.48	43.52 ± 3.64
B	24.43 ± 0.54	46.23 ± 5.93	23.52 ± 2.57	22.78 ± 2.78	22.18 ± 1.81	25.89 ± 1.63
C	15.69 ± 3.96	37.36 ± 2.73	17.17 ± 2.97	17.75 ± 1.89	11.16 ± 2.05	15.88 ± 2.21
D	12.22 ± 2.94	32.60 ± 4.46	14.51 ± 1.81	12.38 ± 0.99	7.76 ± 1.49	08.86 ± 1.44
E	08.54 ± 0.74	10.54 ± 1.18	11.38 ± 0.54	7.47 ± 2.72	03.37 ± 0.77	03.83 ± 1.65
Ampicillin (positive control)	99.56 ± 0.66	99.61 ± 0.67	99.35 ± 0.91	99.64 ± 0.62	99.96 ± 0.07	99.96 ± 0.06
P-value	0.00	0.00	0.00	0.00	0.00	0.00

The extracts of Oolong tea residues were tested against all the bacterial strain as shown in Table 2. For sample A, the highest percentage of inhibition is showed by *S. faecalis* (50.76 ± 1.33), followed by *B. subtilis* (48.97 ± 2.345), *S. typhimurium* (48.77 ± 1.48), *S. aureus* (47.83 ± 2.08), *E. coli* (43.52 ± 3.64) and *P. vulgaris* (35.01 ± 4.04) respectively. However, for sample E (after fifth cycles), the antibacterial properties of the extract were significantly decrease. The highest percentage of inhibition is achieved by S. aureus (11.38 ± 0.54), followed by *S. faecalis* (10.54 ± 1.18), *B. subtilis* (8.54 ± 0.74), *P. vulgaris* (7.47 ± 2.72), *S. typhimurium* (03.37 ± 0.77), and *E. coli* (03.83 ± 1.65) respectively. This shows increasing in times used will influenced the percentage of inhibition of the bacteria.
The ability of the extract to inhibit bacterial growth were decreased after several times of usage due to the increase of pH value of the extract. This is due to high acidic conditions that will inhibit the bacterial growth better than less acidic conditions [18]. In short, there is no significant different between Gram positive and Gram negative bacteria towards the extract.

Furthermore, the effectiveness of the extracts is decreased form the first to fifth cycles might contribute by the heating process during the extraction. In this process, several secondary compounds such as xanthine that are responsible for bacteria inhibition might denatured if introduce under high temperature. Therefore, the amounts of bioactive compound presence in the tea might also reduce and affect the rate of inhibition by the bacteria [19].

3.3. DPPH radical screening activity of oolong tea

DPPH radical screening was conducted to test the antioxidant properties of Oolong tea residues extract. This method is based on the reduction of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical mechanism. Table 3 illustrates the percentage of inhibition of extract of Oolong tea residues on DPPH solution from first to fifth cycle's usage. Meanwhile, Table 4 shows the colour changes in DPPH solution over numbers of used of Oolong tea residues.

Table 3: Percentage of Inhibition of Extract of Oolong Tea Residues on DPPH Solution from First to Fifth Time Used

	% Inhibition	
Times use	Oolong tea	P-value
1 (A)	83.94 ± 0.06	0.000
2 (B)	71.88 ± 0.61	0.000
3 (C)	51.65 ± 0.82	0.000
4 (D)	36.28 ± 1.16	0.000
5 (E)	29.03 ± 1.08	0.000

Table 4: The Colour Changes in DPPH Solution over Numbers of Used of Oolong Tea Residues

No. of Test tube	Samples tested (50g/L)	Colour changes
1	Blank (DPPH)	Purple to purple
2	Ascorbic acid	Purple to light yellow
3	Oolong tea (1st used)	Purple to light yellow
4	Oolong tea (2nd used)	Purple to light yellow
5	Oolong tea (3rd used)	Purple to yellow
6	Oolong tea (4th used)	Purple to cloudy yellow
7	Oolong tea (5th used)	Purple to cloudy yellow

Results from Table 4 shows the colour changes in DPPH solution from first to fifth cycles usage. All tubes showed a changing from purple to yellow in colour. This may cause by the active ingredient such as the flavonoids and polyphenol had reacted with the free radicals of DPPH and it is visually noticeable by the change in the colour from purple to yellow [15]. Flavonoid can scavenge DPPH free radicals by rapidly donating hydrogen atom from hydroxyl group to radicals. In addition, polyphenol which is the main component of tea that play roles as antioxidant agent.

4. Conclusion

As a conclusion, Oolong tea residues has successfully been extracted by using infusion method and water as solvent. The pH value of Oolong tea residues extract was increased from 4.54 to 4.90 after fifth cycles usage. The increasing of pH value leads to poor bacteria inhibition. For the antibacterial test, the highest inhibition is shown by *S. faecalis* (50.76± 1.33) meanwhile the lowest inhibition is show by *P. vulgaris* (35.01 ± 4.04) when tested for sample. However, for sample E, the highest inhibition is shown by *S. aureus* (11.38 ± 0.54) and the lowest inhibition is shown by *E. coli* (03.83 ± 1.65). The percentage of bacteria inhibition is decreases after several times usage of the Oolong tea extract. This is due to secondary metabolites that are responsible for antibacterial properties might been denatured after several times of heating. Meanwhile, Oolong tea residue extracts were proven to have high antioxidants properties due to colour changes (purple to yellow) in DPPH assay at every cycle. In short, the Oolong tea residues possess antibacterial and antioxidants properties even though had been used up to fifth cycles. However, the ability of the Oolong tea residues to act as magnificent tools of antibacterial and antioxidants agent is decreases over time it is used.

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