

# Antioxidant and antibacterial properties of green, black, and herbal teas of *Camellia sinensis*

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## ABSTRACT

**Background:** The role of non-polymeric phenolic (NP) and polymeric tannin (PT) constituents in the antioxidant and antibacterial properties of six brands of green, black, and herbal teas of *Camellia sinensis* were investigated. **Materials and Methods:** Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) were assessed using the Folin-Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, respectively. Minimum inhibitory dose (MID) against Gram-positive *Micrococcus luteus*, *Staphylococcus aureus*, and *Bacillus cereus*, and Gram-negative *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* was assessed using the disc-diffusion method. Teas were extracted with hot water successively three times for one hour each time. The extracts were fractionated using Sephadex LH-20 column chromatography to obtain the NP and PT constituents. **Results:** Extraction yields ranged from 12 to 23%. Yields of NP fractions (70–81%) were much higher than those of PT fractions (1–11%), suggesting that the former are the major tea components. Ranking of antioxidant properties of extracts was green tea > black tea > herbal tea. For all six teas, antioxidant properties of PT fractions were significantly higher than extracts and NP fractions. Extracts and fractions of all six teas showed no activity against the three Gram-negative bacteria. Green teas inhibited all three Gram-positive bacteria with *S. aureus* being the least susceptible. Black and herbal teas inhibited the growth of *M. luteus* and *B. cereus*, but not *S. aureus*. The most potent were the PT fractions of Boh Cameron Highlands and Ho Yan Hor with MID of 0.01 and 0.03 mg/disc against *M. luteus*. **Conclusion:** Results suggested that NP constituents are major contributors to the antioxidant and antibacterial properties of teas of *C. sinensis*. Although PT constituents have stronger antioxidant and antibacterial properties, they constitute only a minor component of the teas.

**Key words:** Extracts, fractions, minimum inhibitory dose, non-polymeric phenolics, polymeric tannins

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## INTRODUCTION

Produced from young leaves of *Camellia sinensis* L. (Kuntz), tea is one of the most popular beverages worldwide. It is cultivated in more than 30 countries worldwide, and of the total amount of tea produced and consumed in the world, 78% is black, 20% is green, and 2% is oolong.<sup>[1,2]</sup> Black tea is consumed primarily in western countries and in south Asian countries such as India and Sri Lanka, whereas green and oolong teas are consumed mainly in east Asian countries such as China, Japan, and Taiwan.

Teas of *C. sinensis* undergo different manufacturing processes. Green tea is produced by steaming (Japan) or panning (China) to prevent catechin oxidation by polyphenol oxidase.<sup>[3]</sup> With no fermentation, green tea leaves retain their green color and almost all of their original polyphenol content. Oolong tea is semi-fermented while black tea is fully fermented.<sup>[4-6]</sup> The different processes of manufacturing give the various teas their characteristic colors and flavors. Oolong tea has an excellent characteristic combining the freshness of green tea and the fragrance of black tea.<sup>[6]</sup>

Green tea contains mainly flavanols or catechins of epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC).<sup>[3,7]</sup> In black tea, the major polyphenols are thearubigins and theaflavins.<sup>[3,8]</sup> The major theaflavins of black tea are

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theaflavin, theaflavin 3-gallate, theaflavin 3'-gallate, and theaflavin 3,3'-gallate.<sup>[9,10]</sup> Theaflavins are orange-red compounds responsible for the astringent taste and coppery color of black tea.<sup>[11,12]</sup> Although thearubigins are most abundant in black tea, their chemical nature and structure are largely unknown. They are water-soluble, acidic, and often rust-brown with structures ranging from dimeric and trimeric to tetrameric, and with molecular weights of 700–2000.<sup>[13]</sup>

Tea polyphenols are well-known for their antioxidant properties. Green tea has greater antioxidant potential than oolong and black teas.<sup>[14-18]</sup> Studies have shown that the strong antioxidant properties of green tea are attributed to catechins of EGCG and EGC.<sup>[19-22]</sup> The three adjacent hydroxyl groups on the B-ring of EGCG, GCG, EGC, and GC are more effective in scavenging free radicals than the two adjacent OH groups of ECG, CG, and EC.<sup>[7]</sup> Black tea is also known to have potent antioxidant properties which are manifested by its ability to scavenge free radicals, inhibit lipid peroxidation, and chelate metal ions.<sup>[9,23]</sup> Although green tea has higher total phenolic content (TPC), free radical scavenging activity, and ferric reducing power, its ferrous ion-chelating ability is poorer than black tea.<sup>[17,18]</sup>

Tea polyphenols are also known for their antibacterial activity. In general, antibacterial activity decreases when the extent of tea fermentation is increased, implying stronger activity in green tea than black tea.<sup>[24,25]</sup> Green tea catechins, particularly EGCG and ECG, have antibacterial activity against both Gram-positive and Gram-negative bacteria.<sup>[26-28]</sup> Green tea can prevent tooth decay by inhibiting oral bacteria.<sup>[29]</sup> The antibacterial activity of black tea has also been reported.<sup>[24-26]</sup>

Although much work has been done on the antioxidant and antibacterial properties on teas of *C. sinensis*, it remains unclear whether these bioactivities are attributed to non-polymeric phenolic (NP) or to polymeric tannin (PT) constituents. This prompted us to compare the antioxidant and antibacterial properties of extracts, NP fractions, and PT fractions of different brands of green, black, and herbal teas of *C. sinensis*.

## MATERIALS AND METHODS

### Tea samples

*Camellia sinensis* green teas (Sea Dyke, Ito En, and Boh), and black teas (Boh Cameron Highlands and Boh Bukit Cheeding) were purchased from the supermarket. Herbal tea of Ho Yan Hor was purchased from a Chinese drug store. The tea samples were stored in a cool dry place

before analysis. Brief descriptions of each of the teas are given in Table 1.

### Extraction

Tea samples were extracted using the hot water method.<sup>[18]</sup> Teas (25 g) were extracted with 250 ml of hot water three times, with continuous swirling at 120 rpm in an orbital shaker, for 1 h each time. The boiling water was allowed to cool throughout the extraction process to mimic tea brewing. After filtration under suction through Whatman No. 1 filter paper, the residues were re-extracted again with

**Table 1: Brief descriptions of the studied teas**

Brand	Description
	Sea Dyke Green Tea is manufactured by Tea Import and Export Co. Ltd. in Xiamen, China. The tea is fragrant with a delicate grassy taste, and is reputed as a health and slimming product.
	Ito En Green Tea is produced and marketed by Ito En Ltd., Tokyo, Japan. The green tea contains natural components, which give it such a distinctive flavor, color, and aroma as it would be freshly brewed in a teapot.
	Boh Green Tea is a recent product of Boh Plantations Sdn. Bhd., Malaysia. It has a refreshing aroma and delicious taste that complement its natural antioxidant properties. Processed according to the traditional Japanese way, the product is intended to provide an affordable green tea of good quality to the market.
	Boh Cameron Highlands is a highland black tea from the Boh tea plantation at Cameron Highlands in Pahang, Malaysia. Available in tea-bags and as loose tea leaves, the tea is renowned for its rich aroma, full-bodied flavor, and distinctive character.
	Boh Bukit Cheeding is a lowland black tea from the Boh tea plantations at Bukit Cheeding in Selangor, Malaysia. Sold in canisters, the garden tea is known for its robust and full-bodied aroma.
	Ho Yan Hor is a well-known Chinese herbal medicinal tea manufactured by Hovid Bhd. in Ipoh, Perak, Malaysia. Of the 23 types of herbs used in its formulation, leaves of <i>C. sinensis</i> are the major ingredient (64%). After 70 years, the herbal tea remains a household name among Chinese families in Malaysia. Available in tea-bags, it is consumed to relieve body heatness, common cold, indigestion, and poor appetite.

250 ml of hot water. The water in the extracts was removed using a freeze dryer. Dried extracts were kept at  $-20^{\circ}\text{C}$  in a freezer for further analysis.

### Fractionation

Tannins were fractionated using Sephadex LH-20 column chromatography.<sup>[30-32]</sup> The crude extract (1 g) was suspended in 10 ml of water and applied onto a chromatographic column (40 × 3 cm) packed with Sephadex LH-20 (GE Health, Sweden) and equilibrated with 100% (v/v) methanol. The column was washed with 200 ml of 100% methanol (v/v) and with 200 ml of 70% acetone (v/v) to obtain the NP and PT constituents, respectively. The fractions were then dried using a rotary evaporator at  $50^{\circ}\text{C}$ .

### Folin-Ciocalteu assay

TPC of extracts was determined using the Folin-Ciocalteu method.<sup>[33,34]</sup> Samples (300  $\mu\text{l}$ , in triplicate) were introduced test tubes wrapped in aluminum foil followed by addition of 1.5 ml of FC reagent (10 times dilution) and 1.2 ml of sodium carbonate solution (7.5% w/v). The tubes were allowed to stand in the dark for 30 min before absorbance was measured at 765 nm. TPC was expressed as gallic acid equivalent (GAE) in mg/g of sample. The calibration equation for gallic acid was  $y = 0.0111x + 0.0148$  ( $R^2 = 0.9998$ ).

### DPPH radical scavenging assay

Antioxidant activity was measured using the DPPH radical scavenging assay.<sup>[34,35]</sup> Different dilutions of the extracts (1 ml) were added to 2 ml of DPPH (5.9 mg/100 ml methanol) in test tubes wrapped in aluminium foil. Absorbance ( $A$ ) was measured at 517 nm after 30 min incubation in the dark. All measurements were made with distilled water as blank. The scavenging ability (%) of the samples was calculated as  $(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$  and calculated as  $\text{IC}_{50}$ , the concentration of sample needed scavenge DPPH free radicals by 50%.  $\text{IC}_{50}$  was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) using the equation:  $\text{AEAC (mg AA/g sample)} = \text{IC}_{50}(\text{AA})/\text{IC}_{50}(\text{sample}) \times 10^5$ . The  $\text{IC}_{50}$  of AA used for calculation of AEAC was 0.00387 mg/ml.

### Disc-diffusion method

Antibacterial activity of extracts and fractions of green, black, and herbal teas were tested against Gram-positive *Micrococcus luteus*, *Staphylococcus aureus*, and *Bacillus cereus*, and against Gram-negative *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. Antibacterial activity was measured using the disc-diffusion method.<sup>[32,36]</sup> Inoculums (100  $\mu\text{l}$ ) were spread evenly onto 20 ml Mueller-Hinton agar set in 90-mm Petri dishes using a sterile cotton swab. Sterilized paper discs (6-mm diameter) were impregnated

with plant samples (2 mg per disc) using a micropipette and firmly placed onto the inoculated agar ensuring even distribution to avoid overlapping of zones. Streptomycin susceptibility discs (10  $\mu\text{g}$ ) were used as positive controls. After incubation overnight at  $37^{\circ}\text{C}$ , the minimum inhibitory dose (MID) or lowest concentration of extract or fraction in mg/disc required to show a zone of inhibition was recorded.<sup>[32,37]</sup>

### Statistical analysis

All experiments were done in triplicate ( $n = 3$ ) and results were expressed as means  $\pm$  standard deviation (SD). Results were analyzed using the Turkey Honestly Significant Difference (HSD) one-way analysis of variance (ANOVA) software developed by Vassar College, New York State, USA. The significant difference was based on  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Extraction and fractionation

Extraction and fractionation yields of the green, black, and herbal teas are shown in Table 2. Yields of extracts ranged from 12% (Ito En Green Tea) to 23% (Ho Yan Hor). Fractionation yields of NP constituents ranged from 70% (Ho Yan Hor) to 87% (Boh Cameron Highland and Boh Bukit Cheeding). Yields of PT constituents were very low in green teas (1–2%), and low in black teas (2–11%) and herbal tea (4%). Results suggested that NP constituents are the major tea components. In a related study on the antioxidant and antibacterial properties of leaves, rhizomes, and inflorescences of three ginger species, yields of NP fractions (66–92%) were also much higher than those of PT fractions (0.5–10%).<sup>[32]</sup>

Hot water was used for extraction since it is the traditional way of brewing tea and previous studies have shown it to be an efficient way of extracting tea. In the extraction of green tea, the yield of hot water extracts was significantly higher than methanol and ethyl acetate extracts.<sup>[21,38]</sup> Similarly, reported that the yield of green tea extract obtained by the hot water extraction (42%) was higher than methanol (41%) and ethyl acetate (25%) extractions. For herbal teas, hot water extraction of microwave-dried tea yielded TPC and AEAC values which were significantly higher than methanol extraction.<sup>[39]</sup> Studies have shown that water temperature is an important factor when extracting tea. Significantly higher yields of hot water than cold water extraction of green tea and stronger radical scavenging activity of oolong tea extracted with hot water of increasing temperature have been reported.<sup>[40,41]</sup> For green, oolong, and black teas, extraction with water at  $100^{\circ}\text{C}$  for 3 min yielded higher total flavanol content than extraction with water at  $60^{\circ}$  and  $80^{\circ}\text{C}$ .<sup>[22]</sup> Commercial green, oolong, and black teas, boiled gently in water for one hour, yielded 15,

17, and 18% of extracts, respectively.<sup>[16]</sup> It has been reported that higher temperatures reduce the polarity of water, thus increasing its extraction efficiency and capability to dissolve less polar compounds.<sup>[42]</sup> Raising the temperature of water also reduces its surface tension and viscosity, which increases the diffusion rate and the rate of mass transfer during extraction.

From the literature, data on the content of tannins for green and black teas are somewhat variable and lower than results of this study. In general, the tannin content in black tea is much higher than in green tea. In the processing of black tea, some 75% of the catechins are converted to thearubigins, while another 10% account for the formation of theaflavins and 15% would remain unchanged.<sup>[43]</sup>

### Antioxidant properties

Antioxidant properties of hot water extracts of green, black, and herbal teas are shown in Table 2. For green teas, TPC and AEAC ranged from 205 mg GAE/g extract and 420 mg AA/g extract in Sea Dyke to 363 mg GAE/g extract and 781 mg AA/g extract in Boh, respectively. For black teas, values were 172 mg GAE/g extract and 269 mg AA/g extract for Boh Cameron Highlands, and 209 mg GAE/g extract and 215 mg AA/g extract for Boh Bukit Cheeding, respectively. Values of Ho Yan Hor herbal tea were 125 mg GAE/g extract and 185 mg AA/g extract, respectively.

Ranking based on TPC of extracts was Boh Green Tea > Ito En Green Tea > Sea Dyke Green Tea ~ Boh Bukit Cheeding > Boh Cameron Highlands > Ho Yan Hor. Ranking based on AEAC of extracts was Boh Green Tea > Ito En Green Tea ~ Sea Dyke Green Tea > Boh Cameron Highlands > Boh Bukit Cheeding > Ho Yan Hor. In general, the antioxidant properties of green tea extracts were stronger than those of black and herbal teas.

Antioxidant properties of fractions of green, black, and herbal teas are shown in Table 2. For all six teas, TPC and AEAC of PT constituents were significantly higher than those of NP constituents. PT constituents in Boh Green Tea yielded the highest values of 1840 mg GAE/g fraction and 3340 mg AA/g fraction, respectively.

Ranking based on TPC of PT fractions was Boh Green Tea > Ito En Green Tea ~ Boh Bukit Cheeding > Sea Dyke Green Tea > Boh Cameron Highlands > Ho Yan Hor. Ranking based on AEAC of PT fractions was Boh Green Tea > Ito En Green Tea > Sea Dyke Green Tea > Ho Yan Hor ~ Boh Bukit Cheeding > Boh Cameron Highlands.

Many studies have shown that green tea has stronger antioxidant properties than black tea.<sup>[14-18]</sup> The main

chemical constituents of green tea are catechins of EGCG, EGC, ECG, and EC.<sup>[3,5,7]</sup> The potent antioxidant activities of catechins in green tea are due to their three adjacent hydroxyl (OH) groups on the B-ring as in EGCG, GCG, EGC, and GC which are more effective in scavenging free radicals than the two adjacent OH groups as in ECG, CG, and EC.<sup>[7]</sup> The content of EGCG and EGC in green tea is much higher than in black tea.<sup>[25]</sup> Although green tea has higher TPC, free radical scavenging activity, and ferric reducing power, its ferrous ion-chelating ability is poorer than black tea.<sup>[17,18]</sup>

The strong antioxidant properties of black tea have been attributed to its chemical components of thearubigins, phenolic acids, catechins, and theaflavins. Theaflavins which impart color, brightness, and astringency to black tea infusion possess potent antioxidant properties.<sup>[9,11]</sup> Theaflavin 3,3'-gallate has been shown to have higher antioxidant activity than EGCG which is the strongest antioxidant among the green tea catechins.<sup>[44,45]</sup> The antioxidant properties of theaflavins have been attributed to their gallic acid moiety.<sup>[46,47]</sup> Theaflavins have more OH groups than catechins since theaflavins are dimers of catechins.<sup>[9]</sup>

This study showed stronger antioxidant properties in PT than in NP constituents. Tannins in the form of

**Table 2: Percentage yield, total phenolic content (TPC), and ascorbic acid equivalent antioxidant capacity (AEAC) of extracts and fractions of *Camellia sinensis* teas**

Type	Brand (country)	Extract/fraction	Yield (%)	TPC (mg GAE/g)	AEAC (mg AA/g)
Green	Sea Dyke Green Tea (China)	HW extract	22	205 ± 9.9 <sup>b</sup>	420 ± 48 <sup>b</sup>
		NP fraction	78	223 ± 11 <sup>b</sup>	418 ± 10 <sup>b</sup>
		PT fraction	1	515 ± 20 <sup>a</sup>	790 ± 39 <sup>a</sup>
	Ito En Green Tea (Japan)	HW extract	12	260 ± 10 <sup>b</sup>	439 ± 29 <sup>b</sup>
		NP fraction	81	231 ± 9.9 <sup>b</sup>	326 ± 18 <sup>c</sup>
		PT fraction	2	551 ± 19 <sup>a</sup>	1170 ± 63 <sup>a</sup>
Boh Green Tea (Malaysia)	HW extract	20	363 ± 25 <sup>b</sup>	781 ± 39 <sup>b</sup>	
	NP fraction	76	351 ± 18 <sup>b</sup>	700 ± 11 <sup>b</sup>	
	PT fraction	1	1840 ± 54 <sup>a</sup>	3340 ± 61 <sup>a</sup>	
Black	Boh Cameron Highlands (Malaysia)	HW extract	18	172 ± 11 <sup>c</sup>	269 ± 27 <sup>b</sup>
		NP fraction	87	218 ± 13 <sup>b</sup>	217 ± 32 <sup>b</sup>
		PT fraction	11	453 ± 61 <sup>a</sup>	507 ± 5.3 <sup>a</sup>
	Boh Bukit Cheeding (Malaysia)	HW extract	16	209 ± 15 <sup>b</sup>	215 ± 22 <sup>b</sup>
		NP fraction	87	160 ± 12 <sup>c</sup>	175 ± 8.3 <sup>c</sup>
		PT fraction	2	537 ± 41 <sup>a</sup>	566 ± 3.8 <sup>a</sup>
Herbal	Ho Yan Hor (Malaysia)	HW extract	23	125 ± 8.4 <sup>b</sup>	185 ± 17 <sup>b</sup>
		NP fraction	70	100 ± 7.6 <sup>c</sup>	123 ± 1.4 <sup>c</sup>
		PT fraction	4	301 ± 18 <sup>a</sup>	582 ± 59 <sup>a</sup>

TPC and AEAC are means ± SD (n = 3). For each column, values followed by the same letter (a–c) are not statistically different at  $P < 0.05$ , as measured by the Tukey HSD test. ANOVA does not apply between different brands of tea. Abbreviations: GAE, gallic acid equivalent; AA, ascorbic acid; HW, hot water; NP, non-polymeric phenolic; and PT, polymeric tannin.

thearubigins, with molecular weights of 1000–40,000 Da, are major constituents of black tea.<sup>[3,13]</sup> The strong antioxidant properties of tannins are due to the large number of phenolic hydroxyl groups and high degree of hydroxylation of aromatic rings.<sup>[48]</sup> Tannins have been reported to be 15–30 times more effective in quenching peroxy radicals than simple phenols.<sup>[49]</sup> Hydrolyzable tannins having galloyl groups exhibited stronger antioxidant effects than flavonoids.<sup>[50]</sup> An increase of galloyl groups, molecular weight and ortho-hydroxyl structure can enhance their antioxidant activity.<sup>[51]</sup> Although tannins may not be absorbed by human due to their big molecular size, they could still exert their antioxidant activity within the digestive tract, and protect lipids, proteins, and carbohydrate from oxidative damage during digestion.<sup>[52]</sup> They can also serve as food preservatives, and as cosmetics and other skin care products.<sup>[53,54]</sup>

### Antibacterial properties

Antibacterial properties of hot water extracts of green, black, and herbal teas are shown in Table 3. All extracts showed inhibitory effects on Gram-positive but not on Gram-negative bacteria. For green teas, extracts of Ito En and Boh showed strong antibacterial activity with MID of 0.06 mg/disc against *M. luteus* and *B. cereus*. Among the three Gram-positive bacteria, *S. aureus* was the least susceptible with Sea Dyke, Ito En, and Boh having MID of 2.0, 2.0, and 1.0 mg/disc, respectively. For black teas, extracts of

Boh Cameron Highlands and Boh Bukit Cheeding inhibited *M. luteus* and *B. cereus* with MID of 0.13 and 0.5 mg/disc, respectively. Interestingly, extracts of black teas showed no inhibitory effect on the growth of *S. aureus*. Extract of Ho Yan Hor herbal tea inhibited *M. luteus* and *B. cereus* (MID of 2.0 mg/disc) with no inhibitory activity against *S. aureus*.

Antibacterial properties of fractions of green, black, and herbal teas are shown in Table 3. All fractions showed inhibitory effects on Gram-positive but not Gram-negative bacteria. For green teas, the NP fraction of Boh green tea displayed the strongest antibacterial activity with MID of 0.06 mg/disc against *M. luteus* and *B. cereus*. Good antibacterial activity of NP fractions of Sea Dyke and Ito En green teas was observed with MID of 0.13 and 0.25 mg/disc, respectively. Again, *S. aureus* appeared to be the least sensitive to the NP fractions of all three green teas. PT constituents fractionated from green teas were insufficient for testing their antibacterial activity. For black teas, the PT fraction of Boh Cameron Highlands strongly inhibited *M. luteus* and *B. cereus* with MID of 0.01 and 0.06 mg/disc, respectively. Similar to extracts, fractions of black teas displayed no antibacterial activity against *S. aureus*. The NP and PT fractions of Ho Yan Hor herbal tea inhibited *M. luteus* and *B. cereus* with no activity against *S. aureus*.

In this study, all extracts and fractions of green, black, and herbal teas showed no antibacterial activity against Gram-negative *E. coli*, *S. typhi*, and *P. aeruginosa*. The inhibition of tea extracts against *P. aeruginosa* and *E. coli* has been reported,<sup>[25,26]</sup> although an earlier study has explicitly reported that tea extracts are not effective against *P. aeruginosa* and *E. coli*.<sup>[27]</sup> The disparity in findings could be due to different strains of bacteria used, and to the different concentrations and types of extracts investigated. Gram-negative bacteria are less susceptible to antibiotics as their outer membrane of lipoproteins and lipopolysaccharides is able to regulate the access of antibacterial agents into the underlying structures.<sup>[54]</sup>

This study showed that green tea extracts inhibited the growth of Gram-positive *M. luteus*, *S. aureus*, and *B. cereus*, with *M. luteus* being the most sensitive. Similar findings have been reported earlier.<sup>[25,26]</sup> Several studies have shown that catechins from green and black teas, particularly EGCG and ECG, inhibited the growth of many bacterial species.<sup>[28]</sup> Contrary to findings from this study, earlier studies have reported that black teas inhibited the growth of *S. aureus*.<sup>[26,55]</sup> Extracts of green tea have been reported to be more effective in inhibiting bacterial growth than black tea.<sup>[24]</sup> In general, antibacterial activity decreased when the extent of tea fermentation increased.<sup>[25,27]</sup>

In this study, the potent antibacterial activity of PT

**Table 3: Antibacterial activity of extracts and fractions of *Camellia sinensis* teas using the disc-diffusion method**

Type	Brand (country)	Extract/fraction	Minimum inhibitory dose (mg/disc)		
			<i>M. luteus</i>	<i>S. aureus</i>	<i>B. cereus</i>
Green	Sea Dyke Green Tea (China)	HW extract	0.50	2.00	0.25
		NP fraction	0.25	-	0.13
	Ito En Green Tea (Japan)	HW extract	0.06	2.00	0.06
		NP fraction	0.25	2.00	0.13
	Boh Green Tea (Malaysia)	HW extract	0.06	1.00	0.06
		NP fraction	0.06	0.50	0.06
Black	Boh Cameron Highlands (Malaysia)	HW extract	0.13	-	0.50
		NP fraction	1.00	-	1.00
	Boh Bukit Cheeding (Malaysia)	PT fraction	0.01	-	0.06
		HW extract	0.13	-	0.50
		NP fraction	1.00	-	0.50
Herbal	Ho Yan Hor (Malaysia)	HW extract	2.00	-	2.00
		NP fraction	0.50	-	2.00
		PT fraction	0.03	-	0.25

Concentration of extracts and fractions used was 2 mg per disc. Abbreviations: HW, hot water; NP, non-polymeric phenolic; PT, polymeric tannin; *M.*, *Micrococcus*; *S.*, *Staphylococcus*; and *B.*, *Bacillus*. PT fractions of all green teas and Boh Bukit Cheeding black tea were not available in sufficient amounts for analysis.

constituents of black and herbal teas against *M. luteus* and *B. cereus* has been demonstrated. The strong and broad spectrum antibacterial properties of tannins have been well documented.<sup>[56-59]</sup> Tannins affect bacterial growth *via* several mechanisms such as inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation.<sup>[60]</sup>

## CONCLUSION

The role of NP and PT constituents in the antioxidant and antibacterial properties of six brands of green, black, and herbal *C. sinensis* teas were investigated. Extraction yields ranged from 12 to 23%. Much higher fractionation yields of NP constituents (70–81%) than PT constituents (1–11%) suggested that the former are the major tea components. In general, the antioxidant properties of green tea extracts were stronger than those of black and herbal teas. For all six teas, antioxidant properties of PT fractions were significantly higher than crude extracts and NP fractions. Extracts and fractions showed no activity against Gram-negative *E. coli*, *S. typhi*, and *P. aeruginosa*. Green teas inhibited all three Gram-positive bacteria with *S. aureus* being the least susceptible. Black and herbal teas inhibited the growth of *M. luteus* and *B. cereus*, but not *S. aureus*. The most potent was the PT fractions of Boh Cameron Highlands and Ho Yan Hor with MID of 0.01 and 0.03 mg/disc against *M. luteus*. Results suggested that NP constituents are major contributors to the antioxidant and antibacterial properties of green, black, and herbal teas. Being non-polymeric, they can be absorbed by the gastrointestinal tract and are therefore orally active. Although PT constituents have stronger antioxidant and antibacterial properties, they constitute only a minor component of the teas.

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