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Antimicrobial effect of Malaysian green tea leaves (*Camellia sinensis*) on the skin microbiota

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ABSTRACT

Aims: *Camellia sinensis* (green tea) is known for its therapeutic properties (anti-inflammatory, anti-oxidative and anti-ageing). The aim of this study was to determine the *in vitro* inhibitory activity of green tea extract on some odorous skin commensal bacteria.
Methodology and results: Tea leaves were collected from MARDI Agro Technology Park, Cameron Highlands. A standardised protocol was used to obtain green tea extract. Aqueous green tea extracts were tested for antibacterial activity by well diffusion method. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assays were performed by broth microdilution assays using green tea extract concentrations from 16 to 0.0313 mg/mL. Green tea extract showed antibacterial activity against skin microbiota. The high antimicrobial effect was achieved against *Micrococcus luteus* with MIC and MBC of 0.125 and 0.25 mg/µL respectively, followed by *Staphylococcus epidermidis* with MIC and MBC of 0.25 and 0.25 mg/µL respectively, *Bacillus subtilis* with MIC and MBC of 0.5 and 0.5 mg/µL respectively and lastly, *Corynebacterium xerosis* with MIC and MBC of 0.5 and 1.0 mg/µL respectively.
Conclusion, significance and impact of study: The results obtained from the study confirm the *in vitro* anti-microbial activity of green tea extracts against skin microbiota. The antibacterial effects of green tea against skin bacteria with its anti-oxidant and anti-aging properties will help in keeping skin healthy, fresh and reducing unpleasant odors.

Keywords: green tea, *Camellia sinensis*, skin microbiota, antimicrobial effect

INTRODUCTION

Green tea is a beverage made from the evergreen plant *Camellia sinensis.* The primary difference between green tea and black tea is in the fermentation process required to produce both tea (Archana and Abraham, 2011). Depending on the tea manufacturing method, tea is divided mainly into green and black tea (Islam *et al*., 2005). Tea prepared from *Camellia sinensis* is used in three forms: fermented black tea, non- fermented green tea and semi fermented oolong tea (Islam *et al.*, 2005). Green tea extract has been reported to have antimicrobial activities against various pathogenic bacteria (Lee *et al*., 2003; Kim *et al*., 2004). Green tea is generally safe, non-toxic and having no side effects after use. It is also believed to be beneficial to health and has a long history of widespread consumption (Hossain and Mahmood, 2014). Since the third century, green tea has been used for medicinal purposes, such as depression, stomach problems, and anxiety (Axelrod *et al*., 2009). A previous study showed that tea has a substantial effect on human health, especially in decreasing blood cholesterol and plays a protective role against cardiovascular diseases and cancer (Lee *et al*., 2003). Green tea extract has strong antioxidant and antimicrobial properties due to its polyphenolic compound contents (Taguri *et al*., 2004). The ingredients in polyphenols, mainly the epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), has an antioxidative property especially by acting as free radical scavengers (Axelrod *et al*., 2009). In addition to that, EGCG can also be used topically to induce anti-aging and anticancer effects in human skin (Chung *et al*., 2003). The exact mechanisms of antibacterial activity of EGCG and ECG are unknown, but it is believed that EGCG disrupts the cell membrane and prevents DNA supercoiling, ultimately leading to the destruction of the bacterial cell

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(Axelrod *et al*., 2009).

Skin microbiota are microorganisms that colonises the human skin. Through aging, human skin microflora undergoes qualitative changes. This can be observed whereby when the *Streptococci*, which are found in infants, are replaced with Coryneform bacteria, which are mainly responsible for odor production (Korting *et al*., 1988). The axillary flora is a stable mixture of *Micrococcaceae*, aerobic *Diphtheroids* and *Propionibacteria*. In moist areas such as the human axilla, *Micrococcus* spp, *Staphylococcus* spp., *Clostridium* spp. and *Bacillus* spp. dominate the resident flora and is associated with pungent axillary odor (Fredrich *et al*., 2013). Numerous bacteria that are found in the normal skin microbiome frequently cause infection in chronic, non-healing wounds, which commonly occur in diabetic patients and the elderly (Sanford and Gallo, 2013). Antibiotics are effective and are used during skin infections. However, they have side effects and resistant clinical isolates might arise due to abuse of the usage of antibiotics (Al-Talib *et al*., 2015). Both antiperspirant and deodorant are used to control body odor by preventing sweat from reaching the skin surface and they also reduce bacteria that causes body odor via antimicrobial ingredients. However, a study has found a link between breast cancer and antiperspirants (Darbre, 2009). Therefore, using of herbal agent which is highly effective and safe might be vital for the reduction of skin bacterial microflora. Currently, green tea extract is used in facemasks, mouthwashes and antiseptic creams (Tiwari *et al*., 2005). Also, tea ointment was successfully used as a topical medicine for impetigo (Chou *et al*., 1999). The use of plant products is increasing worldwide to minimize diseases that may be caused by those bacteria. In Malaysia, no previous study on the inhibitory effects of green tea against skin microbiota has been done. Therefore, in this study, we investigated the antimicrobial activity of the GTE against skin microbiota including *Micrococcus luteus, Staphylococcus epidermidis, Clostridium xerosis* and *Bacillus subtilis*.

MATERIALS AND METHODS

Preparation of green tea extract

Tea leaves were obtained from MARDI Agro Technology Park, Cameron Highlands. Fresh plant leaves were washed under running tap water and ethanol (30-40%). The leaves were cut into pieces and ground into powdery form using pestle and mortar. Crude aqueous infusion was made by adding 20 g of tea leaves which have been previously mashed in a mixer to 1000 mL of sterile ultrapure laboratory grade water by three successive cycles of heating at 80 °C for three minutes (Yam *et al*., 1997; Farooqui *et al*., 2015). Solid materials were removed by filtration using muslin cloth and finally filtered through Whatman No.1 filter paper. The infusions obtained were used immediately.

Bacterial strains

Four types of skin commensal bacterial standard strains (*B. subtilis* ATCC 19659*, M. luteus* ATCC 49732, *S. epidermidis* ATCC 14990 and *C. xerosis* ATCC 373) were used to determine antimicrobial susceptibility and MIC assays. In addition, reference strains *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, were run each time as control for susceptibility and MIC assays.

Antimicrobial susceptibility assays

Agar well diffusion method was used to determine antimicrobial activity of green tea extracts. Two bacterial colonies were inoculated in Tryptic soy broth for 3 h at 37 °C and turbidity was adjusted in phosphate buffered saline to 0.5 McFarland's scale. Using a sterile cotton swab, a small amount of inoculum was spread on Mueller Hinton Agar plates containing 6 mm wells. Twenty microliters of green tea extracts were poured into each well and the plates were incubated at 37 °C aerobically for 24 h. The diameter of the zone of growth inhibition around the wells were measured in millimeters and recorded. Wells containing GTE which showed no inhibition zones were considered as negative results. A twenty microliters solution of vancomycin (30 µg) was added to well as a control.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

Broth microdilution assays was used to determine the MIC and the MBC of the GTE against standard strains as recommended by the Clinical Laboratory Standards Institute (CLSI, 2012). The concentrations of the extracts tested ranged from 16 to 0.0313 mg/mL. This test was performed in sterile 96-well microplates which were loaded with 100 µL of each extracted dilution into each well. Bacterial inoculums 100 µL containing 5 x 10^5 CFU of each microorganism were added to each well. In each plate one well was used for positive control (without extract) and one well was used for negative control (no inoculum added). Plates were aerobically incubated at 37 °C. After incubation for 24 h, bacterial growth was assayed by absorbance measurement at 625 nm. Bacterial growth was also indicated by the presence of turbidity and a pellet on the well bottom. The highest dilution of extract that showed no visible bacterial growth and no turbidity in microbroth dilution assay was considered as MIC.

To determine minimum bactericidal concentration (MBC) of extracts, 100 µL of Mueller Hinton broth from each well of microbroth assay was sub-cultured on MH agar plates after 24 h of initial incubation. MH plates were incubated for another 24 h. The number of surviving organisms was determined. MBC was defined as the lowest extract concentration at which 99.9% of the bacteria were killed. Each experiment was repeated at least twice.

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RESULTS

The antimicrobial effects of GTE against different skin microbiota are summarized in Table 1. All isolates of *M. luteus* were sensitive to the *Camellia sinensis* extract at concentrations of 0.25, 0.5, 1, 2, 4, 8 and 16 mg/mL and exhibited growth inhibition zones ranging from 10 to 26 mm. At 0.125 mg/mL, 25% of *M. luteus* isolates were sensitive, producing inhibition zones of 6–8 mm. *C. xerosis*, *S. epidermidis* and *B. subtilis* were sensitive to *Camellia sinensis* at concentrations of 0.5, 1, 2, 4, 8 and 16 mg/mL and produced inhibition zones ranging from 10 to 35 mm in diameter (Table 1). At the concentration of 0.25 mg/mL of *Camellia sinensis* extract, only *S. epidermidis* was sensitive with inhibition zones ranging from 6 to 9 mm in diameter. None of the bacterial isolates were sensitive to 0.063 mg/mL or less. The most potent inhibitory effect of GTE was observed on *M. luteus* strains with MICs 0.125 mg/mL and a zone of inhibition of more than 25 mm around aqueous extracts. All the strains were found to be sensitive (zone of inhibition ≥ 7 mm) with maximum zone of inhibition obtained for *S. epidermidis* (35 mm). Vancomycin (30 µg/mL) was used as positive control and it exhibited a zone of inhibition of 25 mm. *B. subtilis* and *C. xerosis* had the same sensitivity towards the extract as indicated by the MIC value of 0.5 mg/mL whereas the MIC against *S. epidermidis* was found to be lower at 0.25 mg/mL. MBC values for green tea extracts were two times higher than the corresponding MIC for both *C. xerosis* and *M. luteus* while it showed the same value in *S. epidermidis* and *B. subtilis* (Table 2).

Table 1: Inhibitory zone (mm) of different concentrations of GTE on some skin microbiota.

Skin bacterial microflora	Inhibition zone (mm)											
	GTE concentration (mg/mL)											
	16	8	4	2	1	0.5	0.25	0.125	0.063	0.0313	PC	NC
C. xerosis	26.05 ±1.3	22.05 ±1	20.05 ±1.5	16 ±1.5	11 ±1.5	8.2 ± 1.5	R	R	R	R	Full growth	No growth
S. epidermidis	35.1 ± 0.7	30.1 ± 1	25.7 ±1	21 ±1	11.5 ±1	9 ±2.5	7.5 ±1.5	R	R	R	Full growth	No growth
M. luteus	25.45 ±1	23 ±3	21.15 ± 1	20.5 ±1	15.8 ±2	10.5 ±2	8 ±2	7 ±1	R	R	Full growth	No growth
B. subtilis	25.85 ±1	22. ±2.5	20 ±1.5	15.5 ± 1.5	12 ±1.5	8.5 ± 2.5	R	R	R	R	Full growth	No growth

Table 2: Antimicrobial activity of GTE against skin microbiota.

Bacterial strains	MIC (mg/mL)	MBC (mg/mL)
C. xerosis	0.5	1
S. epidermidis	0.25	0.25
M. luteus	0.125	0.25
B. subtilis	0.5	0.5

Values are mean MICs of GTE ± SD.

DISCUSSIONS

Previous studies have reported the antimicrobial activity of GTE against Gram-positive and Gram-negative bacteria (Cui *et al.*, 2012; Steinmann *et al.*, 2013). However, none of them targeted the effects of green tea on skin microbiota. In this study, we observed that GTE showed antibacterial activity against commensal skin bacterial flora. Up to date, this is the first report providing strong evidence of the antimicrobial activity of Malaysian green tea against skin microbiota. Previous studies showed conflicting reports on the presumptive anti-microbial activity of green tea which could be attributed to different techniques of testing (Reygaert, 2014) and also to the type and origin of the green tea used. Other possible factor might be due to a wide variation in the individual content of catechins in green tea leaves (Axelrod *et al.*, 2009). In this study, to avoid bias and to ensure real and standardized results, we followed the protocols by Clinical and Laboratories Standards Institute (CLSI) for the determination of antimicrobial susceptibility (CLSI, 2012). This study revealed strong inhibitory activity of crude GTE on skin microbiota as this extract produced growth inhibition zones ranging from 9 to 35.8 mm and MIC of 0.125 - 0.5 mg/mL by broth microdilution method, as suggested by previous study (Klancnik *et al.*, 2010). The results of the MIC for GTE against *B. subtilis*, *M. luteus*, *S. epidermidis* and *C. xerosis* based on broth microdilution assays showed that MIC of 0.5 mg/mL could be used as an antibacterial agent against four major bacteria responsible for underarm malodors. These results were confirmed through another report of the *in vitro* growth-inhibiting properties of GTE against underarm bacteria which have been reported to reduce armpit odors (Sharma *et al.*, 2012). The inhibitory activity of GTE on skin microbiota is attributed to polyphenol catechins and particularly EGCG. Different mechanisms have been involved for the antimicrobial activities of tea polyphenol galloylated catechins. EGCG has been shown to cause irreversible membrane disruption in both Gram-positive and Gram negative bacteria and also to inhibit bacterial DNA gyrase preventing DNA supercoiling and leading to bacterial cell death (Gordon and Wareham, 2010). The inhibitory effect of the GTE can be explained also on the basis of preventing attachment of bacteria on the host cell membrane by inhibiting the adhesion of bacteria on host cell surface membranes and acts as a potential anti-adhesive agent (Lee *et al.*, 2009). EGCG has been reported to interact with bacterial outer membrane and

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might prevent the adhesion to mammalian epithelial cells and without any alteration in mammalian epithelial cells (Janecki and Kolodziej, 2010). Another possible mechanism is GTE might affect the activity of dihydrofolate reductase, an essential enzyme which is needed by bacteria to synthesize purine and pyrimidine as well as increase the thickness of the epidermis which can be used as antimicrobial agents (Chung *et al.*, 2003). However, the antimicrobial activity of GTE depends upon presence of different secondary metabolite like hydroxyl group on the active constituents.

In the axilla, a large, permanent population of microorganisms thrives on secretions from skin glands, and producing unpleasant odor (Austin and Ellis, 2003). The classic functional mechanism of deodorants is the removal of cutaneous bacteria by antimicrobial effects of triclosan and aluminum salts (Shahtalebi *et al.*, 2013). However, triclosan and aluminum salts were shown to cause Alzheimer's disease, breast and prostate cancers or induce contact dermatitis. These substances can also cause skin irritations due to the direct topical action of alcoholic or organic substances. Also, the disruption of the integrity of the skin microbiota may have negative effects on the host in terms of health because symbiotic and commensal bacteria participate in immune defense against pathogens (Fredrich *et al.*, 2013). Effective prevention of axillary odor could be achieved by regular use of deodorant and antiperspirants containing antibacterial agents. During the last decade much attention has been given to the antimicrobial activities of medicinal plants and their extracts to be consumed as useful alternatives to synthetic chemical agents. Therefore, replacement by herbal extracts with acceptable antibacterial effects like GTE could reduce the risk of side effects or toxicities due to the extended use of marketed deodorants (Shahtalebi *et al.*, 2013).

In conclusion**,** green tea possesses a potent antimicrobial activity against a variety of skin microbiota and can be included in cosmetic formulations to decrease the bacterial population which is responsible for the pungent bad odor, cellulites, acnes and skin infections. However, additional evaluation is needed to determine the pharmacological property of the active ingredients of the GTE in order to be included in cosmetics.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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