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RESEARCH ARTICLE

Therapeutic value of organic and conventional teas in Sri Lanka against microbial agents

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ABSTRACT

Due to its naturally occurring medicinal ingredients known as polyphenols, tea attracts the interest of the pharmaceutical and scientific disciplines. Besides the established antioxidant activity, many phenolic compounds in tea exhibit significant antimicrobial activity. Environmental factors and crop management substantially influence the amount and activity of polyphenols available in tea leaves. In Sri Lanka, there are six main agro-ecological regions where tea is grown using either conventional or organic management. Present study focused to determine the effects of tea production system and their growing region on polyphenol content and antimicrobial properties of made tea. Fresh leaf samples were collected from randomly selected tea estates each for organically certified and conventional from major tea growing regions in Sri Lanka. They were manufactured into CTC black tea using a miniature system. Polyphenol content was assessed by ISO standard method, while the anti-bacterial and anti-fungal properties were evaluated using disk diffusion technique against Escherichia coli ATCC 25922 and clinical pathogen of Aspergillus niger. Polyphenol content, anti-bacterial and anti-fungal properties were significantly different (P<0.05) between the tea production systems and among the growing regions, where organic tea had higher polyphenol content, anti-bacterial and anti-fungal properties than conventional teas. The Southern region tea had greater anti-bacterial and anti-fungal properties probably due to the high polyphenol content, while it was the lowest in Uva region. In conclusion, this research presents preliminary evidence that Sri Lankan teas grown and managed organically have superior antimicrobial properties over conventional teas. To confirm the findings, more investigation using time series measurement in all tea-growing regions is suggested.

***Keywords**: Anti-bacterial, Anti-fungal, Conventional tea, Organic tea, Polyphenol*

INTRODUCTION

In comparison to water, tea is the most popular beverage worldwide. In all sectors of society, people of all ages enjoy drinking tea. Daily tea consumption worldwide exceeds three billion cups. (Hicks, 2009). The top four producers of tea are China, India, Kenya, and Sri Lanka, with tea being grown in around 30 different nations overall. In terms of global production of tea, Sri Lanka is the fourth-largest producer. (Central Bank Annual Report, 2015). Due to its inherent distinctive qualities and high auction price, Sri Lankan tea, often known as "Ceylon tea," is regarded as one of the best teas in the world. (Kottawa-Arachchi *et al.*, 2011). High grown (up country teas), which are grown in the Nuwara Eliya and Badulla districts and typically grow above 1200 m elevation, low grown (low country teas), which are typically cultivated below 600 m elevation and are primarily found in Galle, Matara, Rathnapura, Kegalle, and Kaluthara districts. The teas grown in Kandy and Matale districts, which are in the middle elevation range (600–1200 m), are referred to as mid-grown or mid country teas (Sandika, 2018).

The recent increase in consumer demand for organic food and beverages on the worldwide market has prompted the promotion of organic agriculture throughout the world (Willer, 2011). Sri Lanka was a leading figure in the production and processing of organic tea. (Hajra, 2001) also ranks third among countries that produce organic tea. (Jayasinghe, 2004). When producing organic tea, synthetic chemicals including pesticides, fungicides, herbicides, growth regulators, and concentrated fertilizers are eliminated. For resistant cultivars, naturally occurring, mined goods, bulky and concentrated organic manures are utilized, along with microclimate regulation, the introduction of biological control agents, or the use of biological products. The goal of organic tea growing is to establish an environmentally friendly plantation that maintains sustainable tea production while protecting the environment and natural habits of the soil, air, and water. (Gaffar and Abdul, 1999).

Modern medical treatments for illnesses are frequently and generally linked to the emergence of side effects. Hence, the usage of plant-based products has expanded globally to minimize negative effects. (Padmini *et al.* 2010). More than 20,000 plant species having therapeutic qualities that cure digestive and respiratory illnesses have been compiled by the World Health Organization (WHO) (Gonçalves *et al.,* 2008). Based on the most recent Sharangi (2009) study of tea's medicinal and therapeutic potentialities, teas have anti-oxidant activity, anti-ulcer impact, anti-inflammatory effect, antibacterial characteristics, anti-cancer properties, and anti-mutagenic activities. Tea leaves contain a variety of nutrients that are good for you, particularly phenolics like catechin, epigallocatechin, and epigallocatechin gallate (EGCG), which are antioxidants, with epigallocatechin (EGC) being the most potent (Owuor and Kwach, 2012). Invading pathogens such as bacteria, fungus, and viruses may be repelled by plants using phenolic chemicals found in their leaves (Chan *et al.*, 2011).

According to research, tea and other products made with organic ingredients are of higher quality than those made with conventional methods. There is evidence that phenolic metabolites are more abundant in organic crops than in conventionally cultivated crops (Bagchi *et al.*, 2015). However, this issue has not been extensively studied in Sri Lanka, especially with regard to the tea crop. Consequently, the purpose of the current study was to identify the effect of crop management system and tea growing region on polyphenol content, anti-microbial properties and made tea quality of Sri Lankan teas.

MATERIALS AND METHODS

Two (02) tea estates per each USDA organically certified (Table 1.) and conventionally managed were selected from each tea growing region (Figure 1.) except Nuwara-Eliya. From each estate, approximately 1kg of fresh leaf samples were collected in triplicates making total of 60 samples.

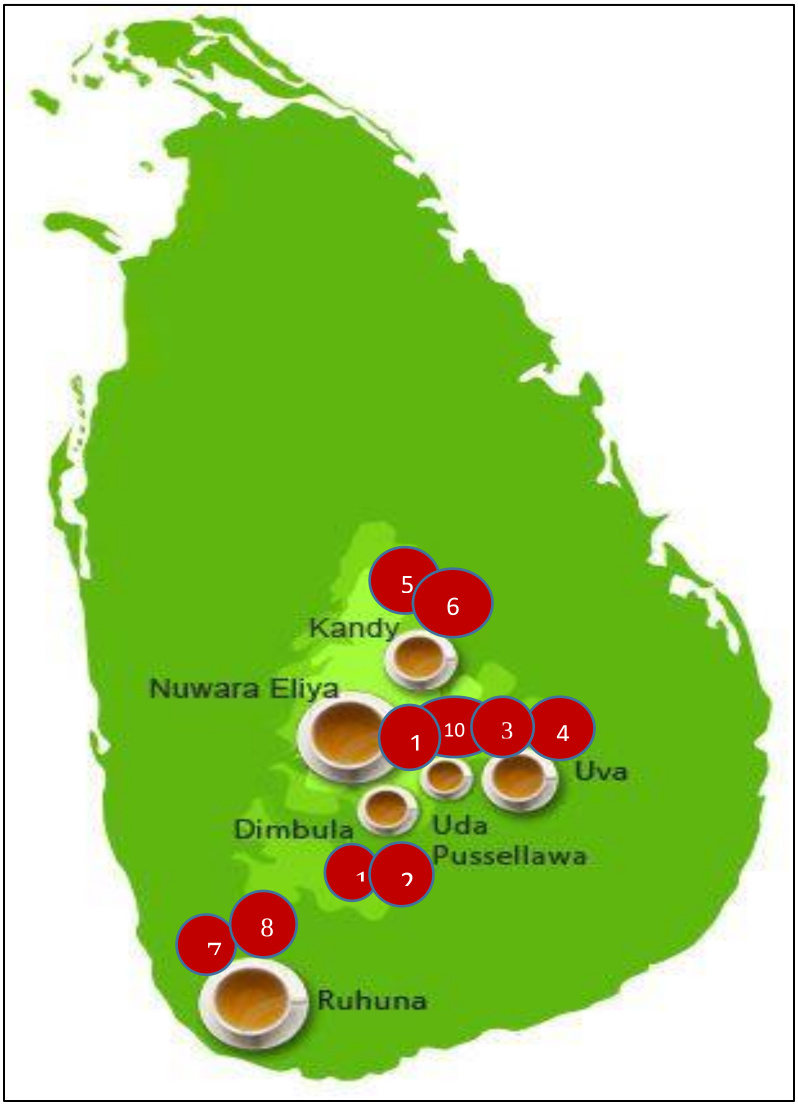

Figure 1: Selected organically certified estates in Sri Lanka

1-Harrington A, 2-Harrington B, 3-Avonleahills, 4-Igalgashinna, 5-James valley, 6-Gampola, 7-Ahinsa, 8-Tsara, 9-Norwood 10-Maha Uwa

Table 1: Organically certified tea estates in different tea growing regions

Tea growing region	Organic certified estates
Dimbula region	Harrington A
	Harrington B
Uva region	Avonleahils, Diyathalawa
	Idalgashinna
Kandy region	James valley
	Gampola
Southern region	Ahinsa, Morawaka
	Tsara, Galaboda
Uda pussellawa region	Norwood
	Maha Uwa

At the Tea Research Institute's Low Country Station in Rathnapura, fresh leaf samples were processed into CTC black tea using small tea processing machinery. At the Research Laboratory of Amazon Trading (Pvt) Ltd, made tea samples were then tested for anti-bacterial and anti-fungal characteristics, while the Analytical Laboratory of Bureau Varitas determined the polyphenol content of tea extracts using the ISO standard technique (14502: PART1:2005E).

Determination of anti-bacterial properties

The anti-bacterial activity against *Escherichia coli* (*E. Coli*) ATCC 25 922 (Bauer *et al.*, 1966) was assessed using the disc diffusion technique on tea extracts that were prepared by mixing 5 g of made tea with 100 mL of boiling water (100^0C). 17.5 g of plate count agar was dissolved in 1000 mL of distilled water and sterilized for 15 min in an autoclave set to 121°C and 15 lbs of pressure. It was then allowed to cool before being placed into sterilized petri dishes. After that, the *E. Coli* ATCC 25 922 was streak-inoculated into agar plates. Filter paper discs with produced tea extracts inside (approximately 5 mm in diameter) were placed on the agar surface, and they were incubated at 37 °C for 18 h. Next, it was decided to evaluate the diameters of the growth inhibition zones (Figure 2)

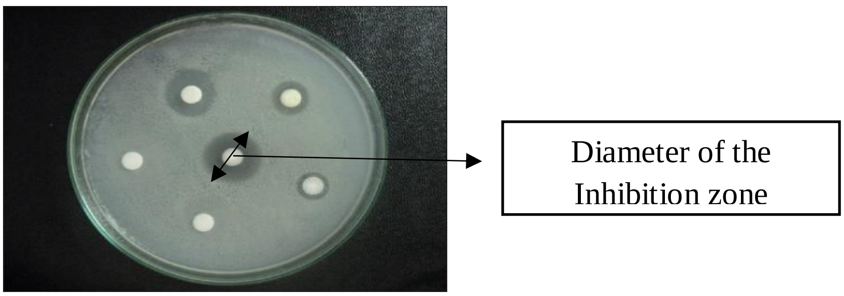

Figure 2. Inhibition zone of disc diffusion technique

Determination of polyphenol content by ISO standard method (14502: PART1:2005E)

By dissolving them in methanol, a gallic acid standard series (25 ppm-100 ppm) was created. Thereafter, 0.2 mL of each gallic acid solution were combined with 0.2 mL of distilled water and 1.0 mL of a Folin-Ciocalteu reagent that had been diluted 10 times. A 7.5% (w/v) Na_2CO_3 solution was added to the solution after 5 min, and the combination was left to remain at room temperature for 30 min. Lastly, distilled water was used as the blank and the produced color was measured at 765 nm. In order to create the calibration curve, absorbance was plotted against the dry mass of gallic acid.

The 0.5 g of made tea sample was brewed in 25 mL of distilled water for 10 min in order to create the 20 000 ppm solution of tea or stock solution. Next, the filtrate was added to a 25 mL volumetric flask and, if necessary, the volume was increased. From this stock solution, a 1000 ppm solution of tea was made, and the above-mentioned gallic acid procedure—which begins with the addition of Folin-Ciocalteu reagent—was repeated using this tea solution rather than gallic acid. The calibration curve was used to read the polyphenol content.

Data Analysis

The analysis of variance (ANOVA) utilizing MIXED models in SAS was used to examine all the parametric data (polyphenol content, anti-bacterial and anti-fungal activities).

RESULTS AND DISCUSSION

There were significant differences in polyphenol content, antibacterial and antifungal properties between organic and conventional crop management systems and tea-growing regions. Moreover, the region where tea is grown and the crop management strategy interacted to affect the polyphenol level (Table 2.).

Table 2: *P* values for the polyphenol content, anti-bacterial and anti-fungal properties of organic and conventional teas in major tea growing regions

	Polyphenol content	**Anti-bacterial properties**	**Anti-fungal properties**
Crop management system	< .0001	< .0001	< .0001
Tea growing region	< .0001	< .0001	< .0001
Crop management system x Tea growing region	0.0002	0.1403	0.1895

Polyphenol content

Significantly high average polyphenol content (11.92 ± 0.87 %) was recorded in organic teas as compared to the conventional teas (11.46 ± 1.09 %). Figure 3 illustrates how crop management practices affect the polyphenol content of black tea. In all tea-growing regions, teas that were grown organically had higher polyphenol contents than teas that were grown conventionally. Bagchi *et al.* (2015) pointed out that current farming methods use significant quantities of pesticides and fertilizers, which can interfere with the plant's normal production of secondary metabolites. This implies that it might be the cause of the current findings.

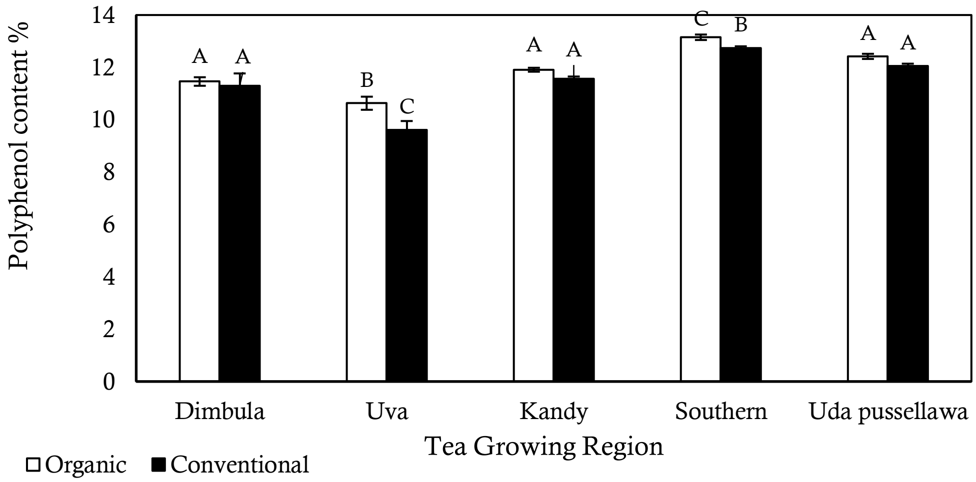

Figure 3: Polyphenol content of organic and conventional teas of different tea growing regions.

Significantly high polyphenol content (12.95 ± 0.22 %) was observed in the southern region while it was the lowest (10.26 ± 0.6 %) in the Uva region. The polyphenol content varies among tea growing regions as Southern region > Udapussellawa region > Kandy region > Dimbula region > Uva region. The highest polyphenol content was recorded in the organic teas grown in Southern region.

Secondary plant metabolites known as polyphenols play a role in both biotic and abiotic stress responses (Pandey and Rizvi, 2009). The climate change has affected the biochemical processes of the plant. The composition of polyphenols, which influences the quality of tea, might fluctuate due to variations in environmental factors. (Kottur *et al.,* 2010). According to Caffin *et al.* (2004), fresh leaves harvested under warmer temperatures exhibited higher catechin gallate concentrations and a greater potential to provide high-quality black tea than fresh leaves harvested under cooler conditions. It may be the reason for the high polyphenol content in southern region than in other regions. Furthermore, according to Owuor *et al.* (1990), minor climatic or geographic changes may affect the chemical makeup and, consequently, the quality of black teas.

The fresh leaf samples used in this research were collected from estates with various altitudes, rainfall patterns, soil types, and other environmental factors. Nevertheless, during the time of sample collection, there was a dry season. It was assumed that a region would experience only minor macroclimatic changes in such a brief period of time. The soils on which the teas were grown varied slightly. However, the fertility gradient of organic tea estates was slightly similar due to the application of compost and plant residues according to the USDA organic regulations. Hence, the main causes of the aforesaid polyphenol fluctuation may be identified as changes in elevation, soil characteristics, and microclimate.

Anti-bacterial properties

In present study, both organic and conventional tea extracts of main tea growing regions were screened for anti-bacterial activity against human clinical pathogen *E coli* ATCC 25922. Significant differences in antibacterial qualities were found between the two primary crop management systems and the major tea-growing regions. However, none of the aforementioned characteristics had an interaction effect on the inhibition zone diameter (Table 2).

All tea extracts examined had an inhibitory effect against clinical pathogen of *E coli* ATCC 25922. Largest inhibition zone diameter (7.73 ± 0.57 mm) was observed in the presence of organic tea extracts (Figure 4).

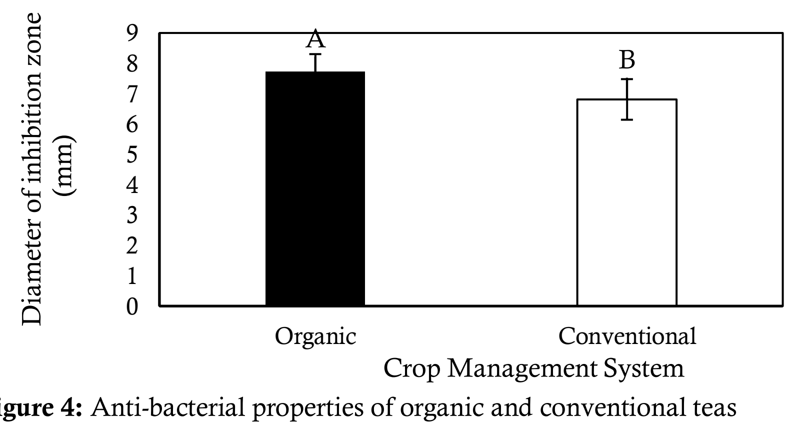

Figure 4: Anti-bacterial properties of organic and conventional teas

The results obtained from present study revealed that tea extracts of southern region had the highest inhibition zone diameter (7.9 ± 0.54 mm), while it was lowest (6.55 ± 0.59 mm) in Uva region (Figure 5). Overall, organic tea extracts in southern region had the highest inhibition zone diameter against *E. coli* bacterial strain.

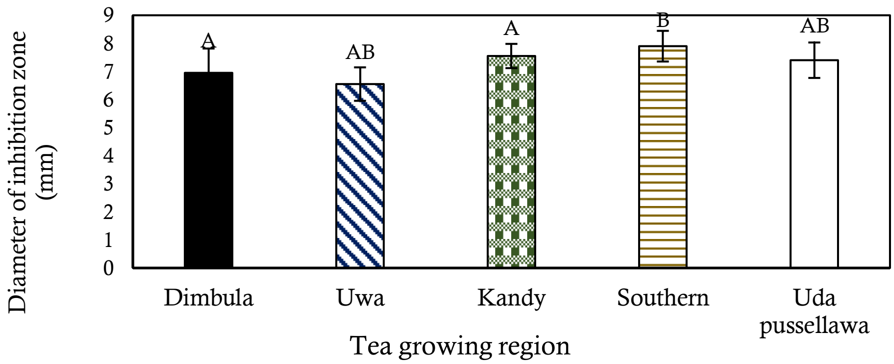

Figure 5: Anti-bacterial properties of organic and conventional teas of different tea growing regions

Due to the ability of their outer lipoprotein and lipopolysaccharide membrane to control the entry of antibacterial drugs to the underlying structures, gram-negative bacteria are less vulnerable to antibiotics. Gram-negative organisms have a variety of lipid molecules in their outer cell membranes that shield the cells from antimicrobial treatments.

The varying degrees of sensitivity of different microorganisms may be due to both the intrinsic tolerance of the microorganisms and the combinations of phytochemical compounds present in the tea extract (Unten *et al*., 1997). The high polyphenol content in tea infusions probably has a significant antibacterial effect on a particular bacterial strain. EGC, EGCG, and ECG are the three main anti-bacterial agents, and there is strong evidence that catechin components in tea extracts are the cause of the observed anti-bacterial activity. Also, it has been proven that black tea, which is a significant source of theaflavins and thearubigins, has antibacterial activities in both *in vitro* and *in vivo.* (Koech *et al.*, 2003).

Several bacterial strains are affected by the phenol catechins, which cause them to produce hydrogen peroxide and change how permeable their membranes are (Ferrazzano *et al*., 2011). This can also be due to the fact that these chemicals might bind to bacterial adhesions, preventing the surface's receptors from being available (Padmini *et al.,* 2010). According to Zhang *et al*. (2006), active substances have the ability to damage the cell wall and membrane, disrupting the permeability barrier and allowing intracellular substances like ribose and sodium glutamate to flow out In addition, they obstruct enzyme function, electron transport, nutrition absorption, protein and nucleic acid synthesis, and growth-inhibiting nutrient transport.

According to Huber *et al.* (2014), polyphenolic substances can obstruct bacterial quorum sensing. The ability to recognize and react to changes in cell population density through regulation is known as quorum sensing. The cytoplasmic membrane of *E. coli* is disrupted by catechin complexes. According to reports, EGC inhibits the activity of the gyrase enzyme by binding to the ATP site of the DNA gyrase b sub-unit of bacteria (Hoshino *et al.,* 1999). The bactericidal effect is based on the generation of hydrogen peroxide from the interaction of EGCG with oxygen. The PTS system, which is in charge of phosphorylating and transporting glucose into the periplasm, is the main glucose uptake mechanism in *E coli*. The attachment of EGC to the cell surface reduced the oxygen consumption rate and immediately inhibited the activity of proteins required for glucose uptake (Motokazu *et al.*, 2015).

Anti-fungal properties

In present study, both organic and conventional tea extracts in all tea growing regions were screened for anti-fungal activity against human clinical pathogen *A. niger*. Anti-fungal properties were significantly different among two main crop management systems and major tea growing regions. However, there was no interaction effect of above-mentioned parameters on inhibition zone diameter (Table .1).

All tea extracts had an inhibitory effect against clinical pathogen of *A. niger.* Largest inhibition zone diameter (7.69 ± 0.47 mm) was observed in the presence of organic tea extracts (Figure 6).

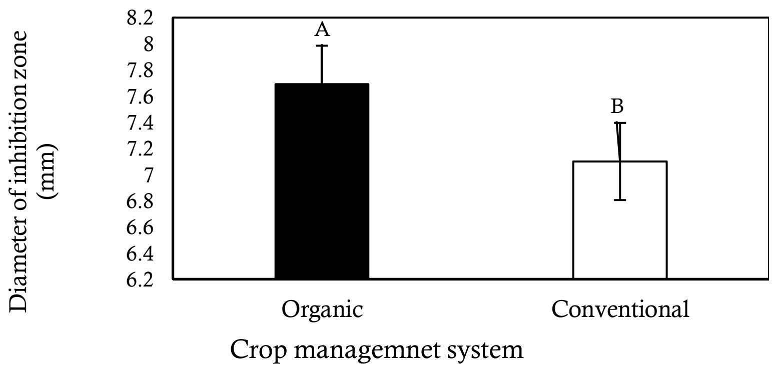

Figure 6: Anti-fungal properties of organic and conventional teas

The results obtained from this study revealed that tea extracts of southern region had the highest inhibition zone diameter (7.92 ± 0.34 mm) while it was the lowest (6.67 ± 0.49 mm) in Uva region (Figure 7). Overall, organic tea extracts in southern region had the highest inhibition zone diameter against *A. niger* fungal strain.

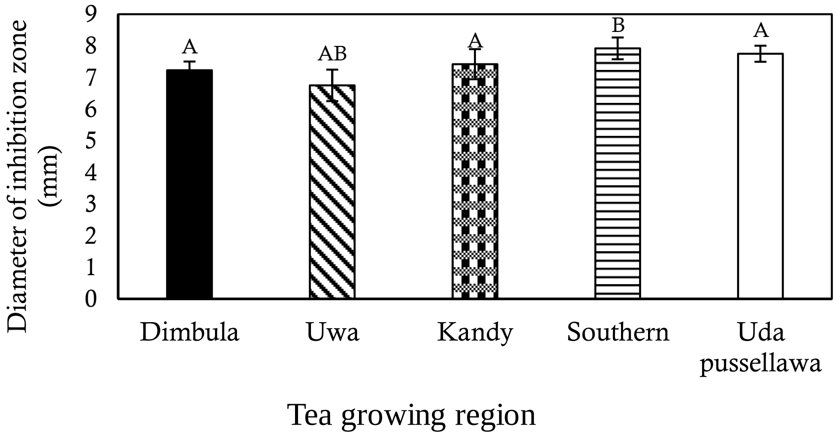

Figure 7: Anti-fungal properties of organic and conventional teas of different tea growing regions

Yang and Jiang (2015) revealed that tea polyphenols directly suppress hyphal growth and spore germination in vitro by changing the synthesis of cell walls, disrupting the plasma membrane, and inducing lysis of hyphae and spores. They bind to membrane ergosterol, which is one of the hypothesized mechanisms for antifungal agents (Kobayashi *et al.,* 1995).

According to established ergosterol-based antifungal action mechanisms, the chemicals may either bind to membrane ergosterol to create pores in this structure or inhibit ergosterol synthesis-related enzymes to lower the amount of that macromolecule (Campoy and Adrio, 2017, Ahmad *et al.*, 2015).

Ergosterol is the target of catechins, which either bind to the sterol or create pores that allow the membrane to become permeable, causing disruption of the cell membrane. Prior metabolic disruption and cell death, EGCG alters the permeability of cell membranes and causes irreversible binding (Borgers, 1980).

CONCLUSIONS

The current research is aimed at evaluating the impact of tea-growing area and crop management system on the polyphenol content and anti-microbial properties of Sri Lankan teas. Results showed that polyphenol content, antimicrobial properties, and the produced tea quality were significantly influenced by both the management system and the growing region. Southern region had the highest polyphenol content while it was the lowest in Uva region. Organic teas had better anti-bacterial properties against clinical pathogen *E coli* ATCC 25922 and anti-fungal properties against clinical pathogen of *A. niger*. Overall, organic teas in southern region had the highest anti-bacterial and anti-fungal properties against human disease-causing bacteria and fungi respectively. Antibacterial and antifungal activities may be mostly due to polyphenol contents. In conclusion, this study has provided some preliminary evidence that organically managed teas have superior pharmacological characteristics to their conventional counterparts, which demands for additional research in order to increase the production of organic tea in the country.

Further research into tea is suggested in order to seek compounds beyond polyphenols for anti-microbial properties. In the current study, disc diffusion technique was used to assess the anti-microbial properties. Antimicrobial compounds or polyphenolic compounds may go through metabolic pathways inside the human body. However, there are no information on the interaction of the related metabolites, hence requires further studies. Thus, an experiment on animals is suggested to determine the mechanism of action of tea polyphenols against pathogenic bacteria and fungus in the human body.

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