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Antifungal activity of crude tea extracts

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The tea polyphenols have been shown to possess many medicinal properties including antifungal activity, but there have been few studies regarding antifungal activity. The antifungal activity of tea polyphenols was evaluated on *Candida albicans* ATCC 90028 and a clinical isolate of *Cryptococcus neoformans* employing the disc diffusion assay. The minimum inhibitory concentration (MIC) of the tea polyphenols against *C. albicans* ATCC 90028 and a clinical isolate of *C. neoformans* was determined. Tea polyphenols showed antifungal activity against *C. albicans* ATCC 90028 and a clinical isolate of *C. neoformans* and both demonstrated an MIC of 1 mg/ml after 24 h. Both fungus were found to be sensitive to tea all tea extracts ($p<0.05$). The inhibition zone diameters significantly ($p<0.05$) and positively correlated to the catechins (EGCG and EGC), total TFs and total TRs. The study reveals the antifungal properties of green, white and black tea products from Kenyan germplasm that may find therapeutic applications in future.

Key words: Tea polyphenols, *Candida albicans, Cryptococcus neoformans.*

INTRODUCTION

Tea is the single largest agribusiness in Kenya and the crop contributes over 26% of all foreign exchange earnings and over 4% of the gross domestic product (Economic Survey, 2005). In Africa, Kenya specializes in black tea production and processing and making the country the third largest producer of tea in the world after China and India (ITC, 2006). Of the numerous tea products in the market, green tea is mainly consumed in China, Japan and the Middle East, while black tea is mostly consumed in India, Sri-Lanka, European countries and regions of Africa. Currently, tea is the leading foreign exchange earner and export commodity amongst agricultural produces in Kenya. In 2010, the tea industry turnover was Kshs 97 billion in foreign exchange after exporting 398.5 million kilograms of made tea (TBK, 2010).

There is a growing body of knowledge on health benefits of tea. This knowledge has however been largely generated from studies using green tea (Carmen et al., 2006). However, little or no work has been carried out on black tea, yet this is the predominant type of tea produced and consumed in Kenya because of a dire paucity of information on the potential health benefits of black tea. Studies have demonstrated that, processed Kenyan black tea have total polyphenols levels that are as high as those found in green teas produced in Asia (Wachira and Kamunya, 2005) and therefore may be as efficacious as green tea in enhancing human health.

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Therefore, there is a need to promptly initiate research on black tea to establish its beneficial effects on human health.

A few studies have focused on the evaluation of antimicrobial activity of black tea, while several scientific studies have been conducted using green tea (Sharangi, 2009). This study therefore is of the first kind aimed at investigating the antifungal activity of aqueous extracts of black, green and white tea products from Kenyan germplasm and green tea products from Chinese and Japanese germplasm against *Candida albicans* ATCC 90028 and a clinical isolate of *Cryptococcus neoformans*.

MATERIALS AND METHODS

Fungi

The test fungi of American Type Culture Collection (ATCC) were sourced from the Kenya Medical Research Institute Centre for Respiratory Disease Research (KEMRI-CRDR) and included *C. albicans* ATCC 90028 and a clinical isolate of *C. neoformans*.

Preparation of tea samples

The tea samples were sourced from Tea Research Foundation of Kenya (TRFK), Timbilil Estate, Kericho (latitude 0° 22'S, longitude 35° 21'E, altitude 2180 m amsl) and processed at the TRFK miniature factory as described by Karori et al. (2007).

Biochemical profiling of the tea extracts based on catechins

A modified high performance liquid chromatography method was used to assay for the tea catechins (Zuo et al., 2002).

Estimation of total polyphenols in the tea extracts

Folin-Ciocalteu phenol reagent method was used to determine total polyphenols in the tea extracts according to ISO (BS ISO 14502-1: 2005(E)).

Analysis of total theaflavins content in the tea sample by flavognost method

Black, green, purple and white teas were also assayed for total theaflavins (TF) using the flavognost method of Hilton (1973).

Spectrophotometric determination of total thearubigins in the tea samples

Total thearubigins (TRs) were determined in the tea samples using the method of Roberts and Smith (1961).

Determination of antioxidant activity of tea

The stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used for determination of free radical scavenging of tea extracts using a modified method of Brand-Williams et al. (1995). The assay is based on the measurement of scavenging ability of antioxidants towards the stable DPPH radical. The percentage inhibition of the DPPH radical was calculated from the absorbance data according to the method of Yen and Duh (1994).

Freeze drying of tea liquors

Tea liquors derived from the processed tea samples were freeze dried according to the method described by Turkmen et al. (2009). Twenty grams (20 g) of the tea sample was mixed with 200 ml of water for 30 min and subjected to continuous hot water extraction at 70°C for 2 h. The resulting water extract was then filtered and subsequently freeze dried using an Edwards Modulyo freeze drier (EF4) to give 2 g of powder, a yield of 20.1%.

Antifungal assays

Fungal isolates of *C. albicans* ATCC 90028 and a clinical isolate of *C. neoformans* were used in the study. The agar disc diffusion method was used to screen for antifungal activities of the tea liquors according to standardize method of the National Committee of Clinical and Laboratory Standards (NCCLS, 2011). The discs used absorbed 0.01 ml of the sample hence the concentration of each sample extract was established.

Minimum inhibitory concentrations

Tea liquors that presented inhibitory properties *in vitro* in the screening activity were evaluated for their minimum inhibitory concentration (MIC) using the disc diffusion test. The MIC was determined as the lowest drug concentration that inhibited growth, as recommended by the National Committee of Clinical and Laboratory Standards (NCCLS, 2011).

Statistical analysis

All determinations were carried out in triplicate and data were subjected to analysis of variance using MSTATC software. The Duncan's multiple range test (DMRT) was used to separate the means.

RESULTS AND DISCUSSION

Antifungal activity

The results of this study showed that different tea extracts had antifungal activity against *C. albicans* ATCC 90028 and a clinical isolate of *C. neoformans* at 1 mg/ml after 24 h (Figure 1). There was no significant difference ($p \leq 0.05$) in the antifungal activity of Kenyan black tea and purple leaf coloured (aerated) tea extracts with the Chinese and Japanese green tea extracts against *C. albicans* ATCC 90028. There was also no significant difference ($p \leq 0.05$) between black tea processed from terminal tea buds of TRFK 301/5 and AHP S15/10 with green tea extracts from Kenyan, Chinese and Japanese cultivars in the antifungal activity. Unaerated tea from purple leaf coloured and white tea extracts did not differ significantly in the antifungal activity with black tea extracts against *C. albicans* ATCC 90028. This

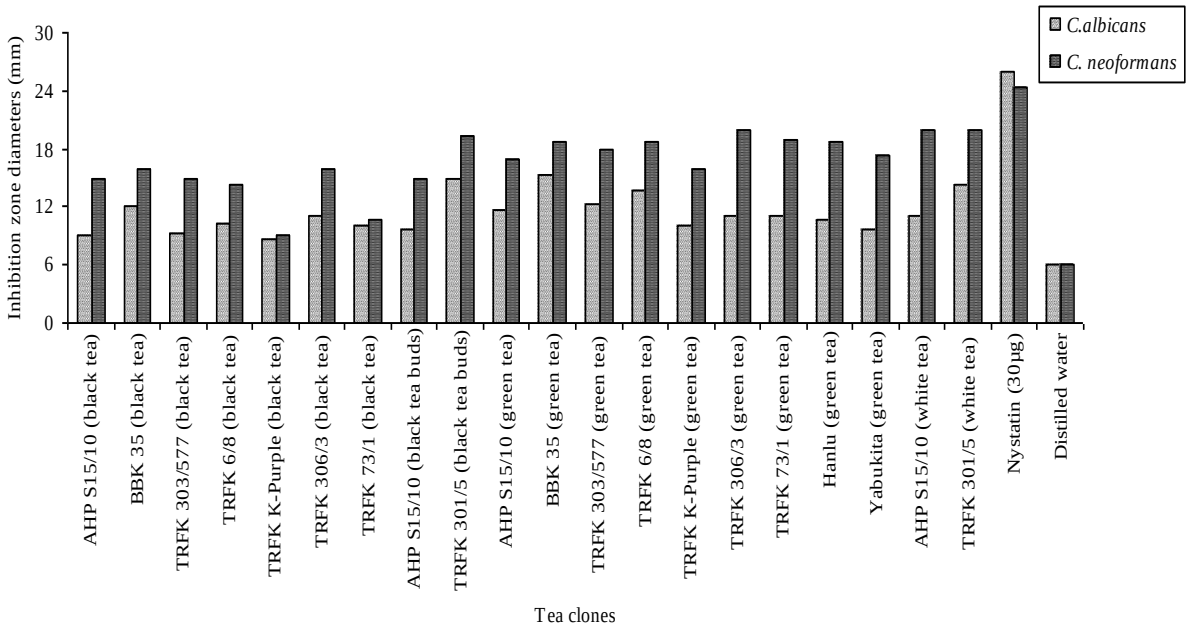

Figure 1. Variation in antifungal activity among different types of tea extracts.

Table 1. Correlation coefficients between inhibition zone diameters (IZDs) and various tea biochemical parameters for *C. albicans* ATCC 90028.

	IZDs	TPP	AA^+	GA	EGC	C	EC	EGCG	ECG	TF	TR
IZDs	1	0.579*	0.479	0.534*	0.426	0.267	0.425	0.514*	0.577*	0.409	0.550*

IZDs, inhibition zone diameters; TPP, total polyphenols; AA^+, antioxidant activity; GA, gallic acid; EGC, epigallocatechin; C, catechin; EC- epicatechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate; Total TF, theaflavins; Total TR, thearubigins; *Correlation significant at p≤ 0.05 level.

corroborated with the results of Sitheeque et al. (2009) who showed antifungal activity of both green and black tea catechins against *C. albicans*.

The antifungal activity shown by tea extracts is probably due to mainly the catechin EGCG and perhaps EGC in green and white tea and theaflavins and thearubigins in black tea. The results on the antifungal activity also indicated that the green tea products as well as products from the purple leaf coloured (unaerated) tea and white tea (silvery tips) products processed from Kenyan tea cultivars exerted the highest antifungal activities. This may indicate that the presence of the hydroxyl moieties at 3', 4', and 5' on the B ring in the catechin and epicatechin molecules is a major contributing factor that contributed to inhibitory activity of both green, unaerated tea from the purple leaf coloured clone and white tea. The contributions of the other catechins might be limited by the fact that only small amounts are presented. This is in agreement with a study reported by Nance et al. (2006) who concluded that antimicrobial activity of catechins is predominantly as a result of the gallic moiety and hydroxyl group member.

A clinical isolate of *C. neoformans* was inhibited by all the different types of tea extracts used in this study (Figure 1). White tea extracts from the Kenyan tea cultivars exhibited the highest antifungal activity against the clinical isolate of *C. neoformans* as compared to black tea, black tea buds, green tea and aerated and unaerated tea extracts from the purple coloured clone. Nystatin (30 µg) was used as a positive standard control while distilled water was used as a negative standard control.

Correlation of biochemical parameters with antifungal activity

The highest antifungal activity also corresponded to the highest total polyphenols content and to antioxidant activity. Several tea polyphenols of black, green and white tea products had significant antifungal activity. These included EC, EGCG, ECG, TFs and TRs. The total polyphenols (r = 0.579*), gallic acid (r = 0.534*), EGCG (r = 0.514*), ECG (r = 0.577*) and total TRs (r = 0.550*) significantly correlated with inhibition zone diameters of *C. albicans* ATCC 90028 (Table 1). The inhibition zones

Table 2. Correlation coefficients between inhibition zone diameters (IZDs) and various tea biochemical parameters for clinical isolate of *C. neoformans.*

	IZDs	TPP	AA^+	GA	EGC	C	EC	EGCG	ECG	TF	TR
IZDs	1	0.765***	0.664**	0.533*	0.486	0.331	0.537*	0.718***	0.620**	0.739***	0.769***

IZDs, inhibition zone diameters; TPP, total polyphenols; AA^+, antioxidant activity; GA, gallic acid; EGC, epigallocatechin; C, catechin; EC-epicatechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate; Total TF, theaflavins; Total TR, thearubigins; *Correlation significant at p≤ 0.05 level; **Correlation significant at p≤ 0.01 level; ***Correlation significant at p≤ 0.001 level.

for clinical isolate of *C. neoformans* correlated significantly with total polyphenols (r = 0.765***), antioxidant activity (r = 0.664**), GA (r = 0.533*), EC (r = 0.537*), EGCG (r = 0.718***), ECG (r = 0.620**), total TFs (r = 0.739***) and total TRs (r = 0.769***) as presented in Table 2. Overly, from this study TPP, EGCG, ECG, GA, total TFs and total TR were identified as the most potent antifungal biochemicals in the assayed teas. Results from this study generally revealed that the inhibition zones were significantly and positively correlated to the catechins (EGCG and EGC), total TFs and total TRs. The results obtained in this study are therefore in agreement with those of Gramza and Korczak (2005), who studied the effects of individual catechins separately and found that EGCG and EGC had the highest antioxidant and antimicrobial activity.

Since medicinal plants produce a variety of substances with antimicrobial properties, screening programs are expected to find out new compounds well suited to the development of new antibiotic drugs. Present findings suggest a potential antifungal activity of tea extracts against *C. albicans* ATCC 90028 and a clinical isolate of *C. neoformans*.

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REFERENCES

Brand-Williams W, Cuvellier ME, Berset C (1995). Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28:25-30.

Carmen C, Reyes A, Rafael G (2006). Beneficial effects of green tea-review. J. Am. Coll. Nutr. 25:79-99.

Economic Survey (2005). Central Bureau of Statistics, Ministry of Planning and National development, Republic of Kenya. pp. 142-157.

Gramza A, Korzak J (2005). Tea constituents (*Camellia sinesis*) as antioxidants in lipid systems. Trends Food Sci. Technol. 16:351-358.

Hilton PJ (1973). Tea in: Snell FD, Ettre LC, Encyclopedia of Industrial Chemical Analysis. 18:455-416.

International Standard (ISO) 14502-1:2005. Determination of substances characteristic of green and black tea - Part 1: Content of total polyphenols in tea - Colorimetric method using Folin-Ciocalteu reagent.

ITC (2006). International Tea Committee, Annual Bulletin of Statistics, Aitken Spence Printing (Pvt) Ltd, Colombo, Sri Lanka. P. 156.

Karori SM, Wachira FN, Wanyoko JK, Ngure RM (2007). Antioxidant capacity of different types of tea products. Afr. J. Biotech. 6:2287-2296.

Nance CL, Williamson M, Cormick T, Paulson S, Shearer S (2006). Epigallocatechin gallate, green tea catechins, binds to the T-cell receptor, CD4. J. Allergy Clin. Immunol. 24:119-124.

National Clinical and Laboratory Standards Institute (NCLSI) (2011). M100-S21, Performance standards for antimicrobial susceptibility testing; Twenty-First informational supplement Wayne, PA.

Roberts EAH, Smith RF (1961). Spectrophotometric measurements of theaflavins and thearubigins in black tea liquors in assessments of quality in teas. Analyst 86:94-98.

Sharangi AB, (2009). Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.): A review. Food Res. Int. 42:529-535.

Sitheeque MA, Panagoda GJ, Yau J, Amarakoon AM, Udagama UR, Samaranayake LP (2009). Antifungal activity of black tea polyphenols (catechins and theaflavins) against *Candida* species. Chemother 55:189-196.

TBK (2010). Tea industry performance in 2010. The Tea Board of Kenya.

Turkmen EN, Sari F, Polat G, Velioglu YS (2009). Antioxidant and antibacterial activities of various extracts and fractions of fresh tea leaves and green tea. Tarim. Bilim. Derg. 15:371-378.

Wachira FN, Kamunya SM (2005). Kenyan teas are rich in antioxidants. Tea 26:81-89.

Yen GC, Duh PD (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. J. Agric. Food Chem. 42:629-632.

Zuo Y, Chen H, Deng Y (2002). Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and puerh teas using HPLC with a photodiode array detector. Talanta 57:307-316.