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In vitro antifungal activity against *Candida* species of Sri Lankan orthodox black tea (*Camellia sinensis* L.) belonging to different agro-climatic elevations

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ABSTRACT

Objective: To investigate the antifungal potential of different grades of Sri Lankan orthodox black tea [orange pekoe, broken orange pekoe fannings (BOPF) and Dust No. 1] belonging to the three agro-climatic elevations (low, mid and high).

Methods: Antifungal activity was assessed *in vitro* using methanolic extracts (300 µg/disc) and agar disc diffusion bioassay technique against three *Candida* species, *Candida albicans* (*C. albicans*), *Candida glabrata* (*C. glabrata*), and *Candida tropicalis*. ketoconazole and itraconazole mixture was used as positive control (10 µg/disc) and methanol was used as the negative control. The minimum inhibitory concentrations were also determined using standard protocols.

Results: None of the extracts were effective against *Candida tropicalis*. Furthermore, orange pekoe grade tea belonging to all agro-climatic elevations did not induce any antifungal activity against *C. albicans* and *C. glabrata* as well. Conversely, Dust No. 1 belonging to all three agro-climatic elevations and low-grown BOPF showed moderate antifungal activity against *C. albicans* and *C. glabrata*. Interestingly, the severity of the antifungal effect varied with agro-climatic elevations. The minimum inhibitory concentrations ranged from 64.00–128.00 µg/mL against *C. glabrata* and 128.00–256.00 µg/mL against *C. albicans*.

Conclusions: Sri Lankan Dust No. 1 and BOPF have marked antifungal activity *in vitro* and offer promise to be used as a supplementary beverage in prophylaxis and during drug treatment in candidiasis.

1. Introduction

Fungi are ubiquitous but few are pathogenic to humans and animals. Over the last three decades, there has been an unprecedented increase in the incidence and diversity of life-threatening invasive fungal diseases including candidiasis[1,2]. However, at present, only few orally active drugs are available for treatment of fungal infections/mycose[1-3]. These are usually expensive (especially in developing countries), and often associated

with undesirable side effects some of which are serious: fever, chills, nausea, dry mouth, metallic taste, alopecia, skin rashes, visual disturbances, muscle weakness, hepatotoxicity and nephrotoxicity[1-3]. Further, there is an increasing clinical and microbial resistance of *Candida* species to several antifungal agents, which becomes a serious problem[2]. As such, there is an imperative need to identify and develop novel antifungal agents which are effective, selective, orally active, safe and also cheap. However, developing an antifungal agent having these characteristics are rather difficult as fungi have metabolic pathways relatively similar to humans[1,2].

In this connection, this study was undertaken to investigate the antifungal activity of three grades of Sri Lankan black tea manufactured by orthodox technique, namely, Dust No. 1, broken orange pekoe fannings (BOPF) and orange pekoe (OP), belonging to three agro-climatic elevations [low grown: below 600 m above

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mean sea level (AMSL); mid grown: 600-1 200 m AMSL; high grown: above 1 200 m AMSL][4]. The antifungal activity was assessed *in vitro* using agar disc diffusion bioassay technique against three *Candida* species: *Candida albicans* (ATCC 90028) (*C. albicans*), *Candida glabrata* (ATCC 90030) (*C. glabrata*), and *Candida tropicalis* (ATCC 13803) (*C. tropicalis*). *Candida* species are opportunistic fungi which cause life-threatening candidiasis, especially in immune-compromised individuals[1,2]. Further, now, candidiasis is one of the most common fungal diseases globally and the most common species responsible for it is *C. albicans*[1,2].

2. Materials and methods

Black tea samples used in this study (Dust No. 1, BOPF and OP) were manufactured using orthodox rotarvane technique with the top most immature leaves and unopened buds of *Camellia sinensis* (*C. sinensis*) plant harvested during August 2015 in three tea factories belonging to three agro-climatic elevations: Mattakale tea factory, Tallawakelle (1 382 m AMSL; high grown, latitude 6°55'59" N, latitude 80°39'00" E), Kirimatiya tea factory, Gampola (600 m AMSL; mid grown, latitude 7°9'36" N, longitude 80°34'12" E) and Kottawally, Galle (0 m AMSL; low grown, latitude 6°2'24" N, longitude 80°12'12" E), Sri Lanka. Tea samples were packed in triplicate laminated aluminum-foil bags and stored at -20 °C until use.

2.1. Sieve analysis

The composition of 'true to size particles' defined for each grade of tea samples was determined in triplicate using a sieve shaker with standard set of sieves with the shaking speed of 50 vibrations/min for 10 min as described by Samaraweera *et al.*[5].

2.2. Organoleptic profile analysis

Typical characters (leaf characteristics, infused leaf characteristics, and liquor characteristics) of each grade of tea samples belonging to the three agro-climatic elevations were organoleptically evaluated by professional tea tasters attached to Sri Lanka Tea Board, Colpitty, Sri Lanka.

2.3. Preparation of methanolic tea extracts

Five grams of each grade of tea belonging to the three agro-climatic elevations were separately extracted into 100 mL of 100% methanol continuously for 5 days with sonication at room temperature (30–32 °C) ($n = 9$). The extracts were filtered through Whatman No. 1 filter paper and the filtrates were evaporated to dryness under reduced pressure in a rotary evaporator.

2.4. In vitro antifungal activity

Each of the methanolic extracts of tea samples belonging to

the three agro-climatic elevations was assessed for the antifungal activity against three pathogenic fungal species, namely, *C. albicans*, *C. glabrata*, and *C. tropicalis*, using standard agar disc diffusion bioassay and Muller Hamilton agar medium as described by Clinical and Laboratory Institute[6]. The concentration of different tea samples used was 300 µg/disc. Ketoconazole and itraconazole mixture (10 µg/disc from each) was used as the positive control and methanol was used as the negative control. The incubation temperature was 37 °C and incubation period was 24 h. At the end of the incubation period, the diameter of the transparent inhibition zone around each disc was measured using a pair of vernier calipers. All the experiments were conducted in triplicate. A tea sample was considered to have an effective antifungal activity if the inhibition growth zone around the disc was equal or greater than 7 mm.

2.5. Evaluation of minimum inhibitory concentration (MIC)

Methanolic tea extracts that demonstrated an effective antifungal activity against *C. albicans* and *C. glabrata* were subjected to the determination of MIC using broth micro-dilution method with slight modifications using Miller-Hamilton broth as the medium and plate reader (to determine optical density of the microbial growth at 600 nm) as described by the National Committee for Clinical Laboratory Standard[6]. Fungal culture concentration inoculated was 0.5 McFarland standards ($\sim 1.0 \times 10^8$ colony-forming unit/mL). The initial concentration of tea samples used was 265 µg/mL, which was serially diluted. Amphotericin was used as the positive control (concentration series used: 2.0–0.004 µg/mL). Results were presented as mean inhibition zone diameter \pm SEM.

3. Results

3.1. Sieve analysis

Sieve analysis showed that more than 80% of tea particles were in the size range specified for each grade: Dust No. 1, 300–500 µm; BOPF 500–800 µm; OP 2000–4000 µm.

3.2. Organoleptic profile

Organoleptic evaluation by professional tea tasters revealed that their leaf characteristics, infusion leaf characteristics and liquor characteristics can be accepted as typical to each grade and their agro-climatic elevations.

3.3. Assessment of in vitro antifungal activities

The results obtained are summarized in Tables 1 and 2. As shown in Table 1, none of the tea extracts was effective against fungal candidiasis pathogen, *C. tropicalis*. Furthermore, extracts of OP grade tea belonging to the three agro-climatic elevations also did not exert any fungal activity against the other tea pathogens, *C. albicans* and *C. glabrata*. On the other hand, mid grown Dust No. 1 [(7.0 \pm

0.2) mm], low grown Dust No. 1 [(8.0 ± 0.4) mm] and low grown BOPF [(7.2 ± 0.4) mm] exhibited mild (in terms of diameter of the inhibitory growth zone) antifungal activity against *C. albicans*. Antifungal activity of positive control (ketoconazole and itraconazole mixture) was (14.6 ± 2.1) mm which was 1.8–2.0 fold higher than the effective tea samples.

With respect to *C. glabrata*, all Dust No. 1 samples belonging to the three agro-climatic elevations [low grown: (8.1 ± 0.6) mm; mid grown: (7.8 ± 0.3) mm; high grown: (8.0 ± 0.6) mm] and BOPF belonging to the low grown elevation [(7.2 ± 0.6) mm] showed moderate antifungal activity. However, the antifungal activity [(15.4 ± 1.5) mm] of the positive control (ketoconazole and itraconazole mixture) was considerably higher (1.9–2.9 fold) than that of the tea samples.

Table 1

In vitro antifungal activity of different grades of Sri Lankan black tea belonging to the three agro-climatic elevations against *C. albicans*, *C. glabrata* and *C. tropicalis*.

Grade of black tea		Antifungal activity (mm)		
		<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. tropicalis</i>
High grown	OP	No	No	No
	BOPF	No	No	No
	Dust No. 1	No	8.0 ± 0.6	No
Mid grown	OP	No	No	No
	BOPF	No	No	No
	Dust No. 1	7.0 ± 0.2	7.8 ± 0.3	No
Low grown	OP	No	No	No
	BOPF	7.2 ± 0.7	7.2 ± 0.6	No
	Dust No. 1	8.0 ± 0.4	8.1 ± 0.6	No
Positive control		14.6 ± 2.1	15.4 ± 1.5	14.6 ± 2.1
Negative control		No	No	No

Data were expressed by mean inhibition zone diameter ± SEM in mm.

MIC values of different black tea extracts against *C. albicans* and *C. glabrata* were shown in Table 2. The lowest MIC value of 64.00 µg/mL was shown by low grown Dust No. 1 and the highest, 256.00 µg/mL was shown by low grown BOPF, whilst both high and mid grown Dust No. 1 samples displayed a MIC value of 128.00 µg/mL. Conversely, positive control had MIC values of 0.50 µg/mL and 0.25 µg/mL for *C. albicans* and *C. glabrata*, respectively.

Table 2

MIC values of different grades of Sri Lankan black tea belonging to the three agro-climatic elevations against *C. albicans* and *C. glabrata*.

Grade of black tea		MIC (µg/mL)	
		<i>C. albicans</i>	<i>C. glabrata</i>
High grown	OP	-	-
	BOPF	-	-
	Dust No. 1	-	128.00
Mid grown	OP	-	-
	BOPF	-	-
	Dust No. 1	256.00	128.00
Low grown	OP	-	-
	BOPF	256.00	256.00
	Dust No. 1	128.00	64.00
Positive control		0.50	0.25
Negative control		-	-

Grade wise, the overall, order of potency of ranking for antifungal activity was Dust No. 1 > BOPF > OP for both *C. albicans* and *C. glabrata*. Agro-climatic wise rank order of potency against *C.*

albicans was low grown > mid grown > high grown for Dust No. 1 and low grown > mid grown = high grown for BOPF. In contrast, agro-climatic wise order of potency against *C. glabrata* was low grown > high grown > mid grown for both Dust No. 1 and BOPF.

4. Discussion

This study examined the antifungal activity of three grades of Sri Lankan orthodox black tea (Dust No. 1, BOPF, and OP) belonging to the three agro-climatic elevations (low, mid and high) of the country[4]. The *in vitro* technique used to ascertain the antifungal activity was a widely employed inexpensive and well-established method[7,8]. Methanolic extracts were employed since they were the most common solvent used in investigation of plant products for anti-fungal activity[9,10]. The tea samples used were typical and representative to each grade and agro-climatic elevation in the country (in terms of sieve analysis, organoleptic properties and external appearance)[11,12], unblended and garden fresh. We believe that these characteristics make this study superior and more meaningful. Pharmacological and physiological activities of black tea are known to vary with several factors including the country of origin, agro-climatic elevation, processing method, harvesting season, grade of tea or particle size[13,14].

The results clearly showed for the first time that Sri Lankan orthodox black tea Dust No. 1 and BOPF had promising antifungal activities against *C. albicans* and *C. glabrata* but not against *C. tropicalis*. Interestingly, the antifungal activity was similar against both these two *Candida* species. However, antifungal activity was found to be significantly (about 50%) lower than the referenced drug. Nevertheless, antifungal activity of the tea extract is noteworthy since we have used the pure and active form of the positive drug whilst the tea samples used are crude methanolic extracts. In contrast, somewhat surprisingly, none of the OP grade teas belonging to the three agro-climatic elevations exhibited any antifungal activity against all the three *Candida* species tested. Our previous studies on anti-bacterial activity of these selected tea grades showed least activity in OP grade[15]. However, Dust No. 1 and BOPF grades also showed anti-bacterial activity, although anti-fungal activity was absent in this study.

Among the grades of tea tested, the order of potency was found to be Dust No. 1 > BOPF > OP. In agreement with this study, a similar order of potency was evident with anti-rheumatoid arthritic activity[16], sunscreen[17] or antibacterial activity[15]. This could be due to differences in particle size of the different grades of tea used in this study. Dust No. 1 has the smallest particle size while OP has the largest. Smaller the particle size larger the surface area to volume ratio[18] and greater the release of active phytoconstituent[16,17], thus increases the severity of the pharmacological activity. Alternatively, the ranking order of potency, agro-climatic elevation wise, was different against the two *Candida* species: against *C. glabrata* it was low grown > mid grown > high grown for Dust No. 1 and low grown > mid grown = high grown for BOPF; and against *C. albicans* it was low grown > high grown > mid grown for both Dust No. 1

and BOPF. These differences in potencies between the grades and the agro-climatic elevations could be due to differences in the profile of phytoconstituents evident amongst the grades[11,12].

The polyphenols, catechins and theaflavins isolated from Sri Lankan black tea, although the grades are not specified, are shown to have antifungal activities against 5 *Candida* species: *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, and *Candida tropicalis*[8]. We have previously shown that the three grades of tea used in this study contained substantial amounts of catechin and theaflavins[11,12]. Accordingly, it is not unreasonable to presume that the antifungal activity in this study is mediated via these two polyphenols. Albeit, it is presently unknown why all the three grades of black tea investigated were ineffective against *C. tropicalis* in spite of the presence of these polyphenols. Satheequ *et al.*[8] have shown that catechins and theaflavins extracted from Sri Lankan black tea (grade or agro-climatic elevations were not specified) have anti-fungal activity against *C. tropicalis*.

Currently, available antifungal agents mediate their therapeutic actions mainly by inhibiting ergosterol synthesis (ergosterol is a vital steroid in fungal cell walls) and/or disrupting the fungal cell walls[1-3]. Since black tea contains complex polyphenolic constituents such as catechins, theaflavins and thearubingins and many more other compounds[11,12,19], it is possible that the antifungal activity may be mediated by disruptions of the integrity of the fungal cell wall as reported for some antifungal drugs[1-3].

On the basis of our results, it is concluded that Sri Lankan Dust No. 1 and BOPF grades of orthodox black tea have promising antifungal activities at relatively low concentrations against *C. albicans* and *C. glabrata*. The results also hint that these Sri Lankan black teas offer high promise as a supplementary beverage both in prophylaxis and during drug treatment against candidiasis. Currently, tea is the most consumed beverage besides water and it is nontoxic even with high regular consumption[19].

Conflict of interest statement

We declare that we have no conflict of interest.

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