ANTI-CANDIDA ACTIVITY OF CATECHINS ISOLATED FROM FRESH TEA FLUSH, MATURE TEA LEAVES AND GREEN TEA

A.A.K. Karunathilake[1], S.R.W.A.B.M.N.S. Kandepola[1], G.J. Panagoda[2], V. Thevanesam[3], N.S. Kumar[1] and B.M.R. Bandara[1*]

[1]Department of Chemistry, Faculty of Science, [2]Division of Microbiology, Faculty of Dental Sciences, [3]Department of Microbiology, Faculty of Medicine, University of Peradeniya

Introduction

Candida albicans plays an important role in candidosis, denture stomatitis, periodontitis, vulvovaginitis and candidemia. The proportion of infections due to other *Candida* species such as *C. parapsilosis*, *C. glabrata* and *C. guilliermondii* is also significant (Pfaller *et al.*, 1998). The toxic effects of antimycotics used in the treatment of *Candida* infections and the appearance of antimycotic-resistant *Candida* strains point to the need for developing highly effective and safe alternatives to conventional antimycotics (Hirasawa *et al.*, 2004). Previous studies reveal that catechins present in green tea (Hirasawa *et al.*, 2004) and black tea (Sitheeque *et al.*, 2009) have anti-Candida properties; tea (*Camellia sinensis*) is consumed worldwide and is a major export crop in Sri Lanka. We describe here the isolation of catechins from fresh tea flush, mature tea leaves, green tea and green tea dust and evaluation of their anti-Candida activity against six *Candida* species.

Materials and Methods

Tea flush was collected from the tea estate of the Tea Research Institute at Upper Hanthane (TRI 2023, TRI 2025). Two green tea samples were obtained from Stassen Exports Ltd. (Stassen green tea-large leaves, green tea dust-powder form). Clinical isolates of six Candida species (*C. albicans, C. sake*, *C. guilleiermondi*, C. dublinensis, *C. rugosa*, and *C. parapsilosis*) were obtained from the culture collection of the Faculty of Dental Sciences, University of Peradeniya.

Crude catechin mixture (CCM) was isolated by separately extracting fresh tea flush (TF), mature tea leaves (ML), Stassen green tea leaves (GL) and green tea dust (GD) with 70 % aqueous methanol followed by partitioning the methanol extract with dichloromethane and then with ethyl acetate. Ethyl acetate extract from each tea sample was concentrated and freeze-dried to obtain CCM-TF, CCM-ML, CCM-GL and CCM-GD. CCM-TF was fractionated using high speed counter-current chromatography to obtain epigallocatechin gallate (EGCG) at a solvent pumping rate of 1.5 mL min^{-1} and a centrifugation of 800 rpm for 5 h on head-to-tail mode and 3 h on tail-to-head mode.

Agar well diffusion assay was performed to determine the anti-Candida activity of catechins; well diameter was 9 mm. Cell suspensions

Table 1. Width of the inhibition zone around the wells containing CCM samples on each Candida plate in the agar well diffusion assay

Species	Strain No	CCM Consentration /ppm	Width of the inhibition zone / mm			
			CCM-TF	CCM-ML	CCM-GL	CCM-GD
C. albicans	C17	1000	5.0	5.0	3.0	4.0
		2000	3.0	3.0	5.0	4.0
		2500	8.0	7.5	7.5	6.0
		3000	8.0	6.0	9.0	7.0
		4000	5.5	5.5	5.0	4.0
		5000	4.0	5.5	5.5	5.5
C. sake	C04	1000	8.0	4.0	5.0	6.0
		2000	4.5	4.5	5.0	5.5
		2500	10.1	10.5	12.0	11.5
		3000	12.0	10.0	11.5	11.0
		4000	10.0	10.0	8.0	7.5
		5000	10.5	10.5	11.0	10.0
C. parapsilosis	C09	1000	5.0	4.0	5.0	6.0
		2000	6.0	6.0	5.0	6.0
		2500	9.0	8.0	11.0	10.0
		3000	10.5	9.5	9.5	9.5
		4000	9.0	6.5	6.0	5.0
		5000	9.5	8.5	7.0	8.0
C. dublinensis	C03	4000	10.0	7.0	10.0	9.5
		5000	9.5	7.0	8.5	9.5
C. guilleiermondi	C05	4000	15.0	10.5	14.0	11.0
		5000	16.5	13.5	13.0	14.5

adjusted to the turbidity of a 0.5 McFarland standard were used to inoculcate by pour plate method. Agar plate dilution method based on the BSAC standard (Andrews, 2001) was performed to analyze the minimum inhibitory concentration (MIC). Assays were performed on Muller-Hinton agar with one-day-old growths of microorganism. Plates were incubated aerobically at 36 to 37 °C and read at 20 to 24 h.

Results and Discussion

The yield (w/w %) of CCM varied with the tea sample: 17 % (from green tea leaves), 15 % (green tea dust), 1.6-2.3 % (fresh tea flush), 0.3-0.7 % (3rd matured leaf) and 0.2-0.3 % (4th matured leaf).

All the six *Candida* species showed sensitivity to the four CCMs. In the agar well diffusion assay (Table 1), *C. sake* and *C. guilleiermondi* displayed the highest sensitivity while *C. albicans* had the least. In the agar plate dilution method (Table 2), *C. guilliermondii* showed high susceptibility to CCM having an MIC of 256 ppm for CCM-GL. Again *C. albicans* was the least sensitive organism against all the CCMs examined.

The activity of EGCG was less than that of the CCM implying that there might be highly active compounds present in CCM other than EGCG or there may be a synergetic effect.

Table 2. MIC of four different CCM samples and EGCG on different Candida strains

Strain		MIC value /ppm				
Species	Ref. No.	CCM-TF	CCM-ML	CCM-GL	CCM-GD	EGCG
C. albicans	C17	>2048	>2048	>2048	>2048	>2048
	C18	>2048	>2048	>2048	>2048	>2048
C. sake	C04	1024	256	1024	512	1024
C. guilleiermondi	C05	512	512	256	512	1024
	C06	1024	512	256	512	1024
C. dublinensis	C03	>2048	1024	256	2048	1024
C. rugosa	C19	>2048	1024	1024	1024	512
C. parapsilosis	C01	2048	>2048	1024	2048	>2048
	C02	512	256	2048	512	>2048
	C08	1024	>2048	512	>2048	2048
	C09	>2048	>2048	1024	>2048	>2048
	C10	1024	2048	1024	1024	>2048
	C11	512	512	1024	1024	512
	C12	1024	2048	512	2048	>2048
	C13	>2048	>2048	>2048	>2048	>2048
	C14	1024	512	1024	512	>2048
	C15	1024	>2048	512	>2048	2048
	C16	2048	512	1024	512	>2048

Conclusion

Tea catechins isolated from fresh tea flush, mature tea leaves, green tea, or green tea dust show considerable activity against important Candida species, C. guilleirmondi and C. sake being the most sensitive.

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