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Evaluation of the Effects of Hot Spring Water on Growth of Candida Albicans

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Abstract: ***Long prevailing belief of getting relief from skin ailments by dipping in hot spring pools was categorically investigated in terms of inhibition of growth of Candida albicans (clinical isolate and reference strain) in addition to consequences on 'germ tube' formation. Excepting rainy season (likely to be for dilution by rain water), water from Taptapani was significantly more efficacious than that from Bakreswar, though both showed promising results. The specific reasons (presence of ions etc) need to be evaluated further along with possible application on clinical settings.***

Keywords: Hot spring water, Candida albicans, Germ tube, Taptapani, Bakreswar

1. Introduction

Since ancient times, bathing in hot springs was believed to cure skin maladies[1] including fungal infections. However, scientific introspect into the matter remained lacking. Candida albicans, being the commonest mycotic agent infecting humans, and associated with acute exacerbations of atopic dermatitis[2], needed to be challenged by growing in hot-spring water towards assessing the effects.

2. Objective

The purpose of the present study was to evaluate and compare the in vitro effects of hot spring water, collected from two sources (Bakreswar, 23^0 53' 0" North, 87^0 22' 0" East. Birbhum, WB and Taptapani, 19^0 30' 0" North, 84^0 24' 0" East, Ganjam, Orissa) on C. albicans in terms of growth rate and germ tube formation to appraise its usefulness in the management of some infective mycoses in a scientific manner, confirming the activity of a biocide.

3. Methods

1. Collection of water from hot springs in different seasons followed by filtration through nitrocellulose (Millipore®) membrane filters having pore size 0.25µ (smaller than that of the smallest bacteria as well as fungus). The composition was assessed using method as described in APHA (20th edition).
2. Determination of the colony numbers of C. albicans (clinical isolate and reference strain MTCC 227) by spreading standard inoculums (containing 2 X 10^3 CFU / ml) as lawn culture on Sabouraud Dextrose Agar (SDA) media containing hot spring water (50% & 100%) and distilled water (as control) after aerobic incubation for 24 hrs at 37^0C[3]. Three sets of experiments were made and the average value was considered.
3. Observation of the percentage of germ tube formation (in HPF counting 100 cells) by C. albicans grown on those media after growing in human serum at 37^0C for 3 hrs[4].
4. Statistical test used - Statistica.

4. Result

All the parameters tested had shown encouraging results confirming that among those two sources, water from Taptapani was more effective or had naturally got more powerful anti-candidal activity. In summer and winter they showed better efficacy and may be for dilution and decrease in pH by rain water (see tables).

Table 1: Physical & chemical nature of the samples from Taptapani (T) and Bakreswar (B)

Season	Summer		Rainy		Winter		mg/L	T	B
Source	T	B	T	B	T	B	Na^+	133.9	79.9
Temperature (^{0}C) (When collected)	68-72	80-82	60-62	72-74	44-48	60-62	K^+	1.8	2.6
pH	8.6	7.9	7.9	7.2	8.2	7.6	$SO_4^=$	25	63
Taste	Metallic						Cl^-	7.8	9.7
Smell	Sulphureous						$PO_4^=$	<0.01	<0.01

Table 2: Colony count of C. albicans after 24 hrs of incubation in different seasons

A. Summer (Strain A- Clinical Isolate, Strain B-MTCC 227)

Water used	% of hot spring water used	Strain of C. albicans	Cfu (no. X 10^2/ml)			Average value
			Set-1	Set-2	Set-3	
Distilled water	0%	A	19.64	19.68	19.66	19.66
		B	19.68	19.61	19.64	19.67
Bakreswar	50%	A	7.21	6.93	7.01	7.05
		B	6.95	7.13	7.19	7.09
	100%	A	6.82	6.65	6.92	6.79
		B	6.72	6.81	6.71	6.75
Taptapani	50%	A	5.72	5.1	4.88	5.23
		B	5.22	5.33	5.21	5.25
	100%	A	3.81	4.32	4.01	4.04
		B	4.05	4.25	4.02	4.11

B. Winter (Strain A- Clinical Isolate, Strain B-MTCC 227)

Water used	% of hot spring water used	Strain of C. albicans	Cfu (no. X 10^2/ml)			Average value
			Set-1	Set-2	Set-3	
Distilled water	0%	A	19.64	19.68	19.66	19.66
		B	19.68	19.61	19.64	19.67
Bakreswar	50%	A	7.72	7.17	7.25	7.38
		B	6.99	7.15	7.33	7.16
	100%	A	7.15	6.95	7.25	7.11
		B	6.91	6.84	6.99	6.91
Taptapani	50%	A	5.18	5.62	5.01	5.27
		B	5.26	5.4	5.33	5.33
	100%	A	3.92	4.05	4.19	4.05
		B	4.01	4.22	4.09	4.11

C. Rainy (Strain A- Clinical Isolate, Strain B-MTCC 227)

Water used	% of hot spring water used	Strain of C. albicans	Cfu (no. X 10^2/ml)			Average value
			Set-1	Set-2	Set-3	
Distilled water	0%	A	19.64	19.68	19.66	19.66
		B	19.68	19.61	19.64	19.67
Bakreswar	50%	A	17.18	17.55	17.91	17.76
		B	16.99	17.61	17.65	17.42
	100%	A	16.05	16.35	16.01	16.13
		B	16.19	16.22	16.11	16.17
Taptapani	50%	A	17.17	18.25	17.82	17.78
		B	17.87	17.01	17.76	17.55
	100%	A	15.88	16.15	16.07	16.02
		B	16.08	15.89	15.97	15.98

NB: Differences were significant in Summer & Winter seasons only

Table 3: Germ tube formation of C. albicans in different seasons

A. Summer (Strain A- Clinical Isolate, Strain B-MTCC 227)

Water used	% of hot spring water used	Strain of C. albicans	% of cells with Germ tubes			Average value (%)
			Set-1	Set-2	Set-3	
Distilled water	0%	A	32	33	25	30
		B	31	28	31	30
Bakreswar	50%	A	19	18	17	18
		B	20	21	17	19.33
	100%	A	16	16	15	15.67
		B	15	14	15	14.67
Taptapani	50%	A	13	14	14	13.67
		B	14	15	13	14
	100%	A	11	11	12	11.33
		B	12	12	11	11.67

B. Winter (Strain A- Clinical Isolate, Strain B-MTCC 227)

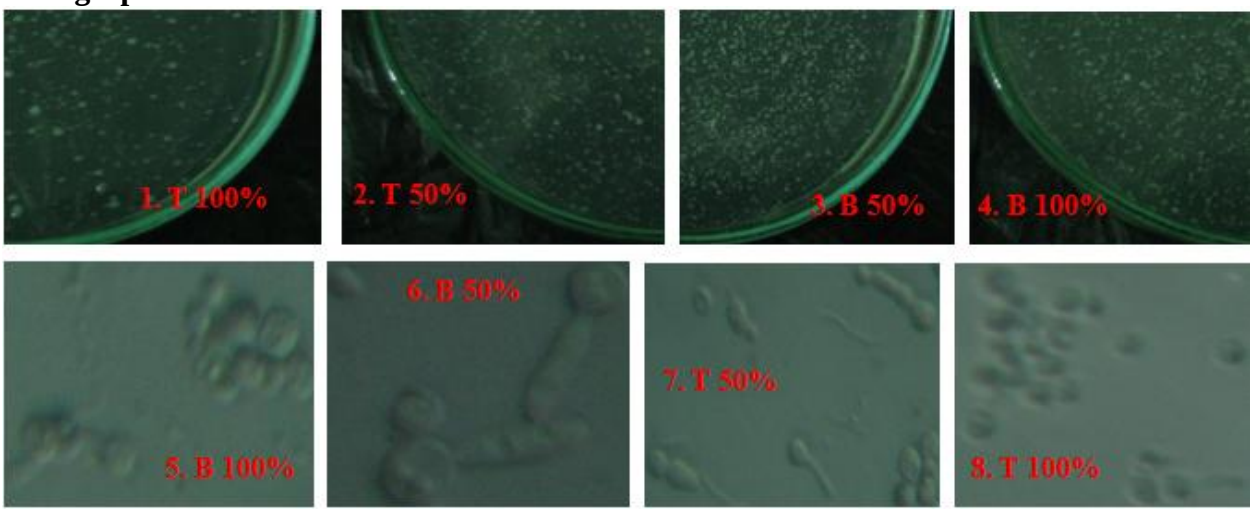
Water used	% of hot spring water used	Strain of C. albicans	% of cells with Germ tubes			Average value (%)
			Set-1	Set-2	Set-3	
Distilled water	0%	A	32	33	25	30
		B	31	28	31	30
Bakreswar	50%	A	18	18	17	17.67
		B	20	20	18	19.33
	100%	A	15	16	15	15.33
		B	15	15	14	14.67
Taptapani	50%	A	13	13	12	12.67
		B	13	13	13	13
	100%	A	11	9	10	10
		B	11	12	11	11.33

C. Rainy (Strain A- Clinical Isolate, Strain B-MTCC 227)

Water used	% of hot spring water used	Strain of C. albicans	% of cells with Germ tubes			Average value (%)
			Set-1	Set-2	Set-3	
Distilled water	0%	A	32	33	25	30
		B	31	28	31	30
Bakreswar	50%	A	29	28	27	28
		B	30	23	26	26.33
	100%	A	26	26	25	25.67
		B	25	24	25	24.67
Taptapani	50%	A	28	28	27	27.67
		B	24	25	24	24.33
	100%	A	26	26	27	26.33
		B	22	22	21	21.67

NB: Differences were significant in Summer & Winter seasons for 100% water from Taptapani only

5. Photographs



Legends- **T**: Taptapani, **B**: Bakreswar, **%**: percentage of water used
Upper panel shows colonies of C. albicans, Lower one shows Germ Tube formation

6. Discussion

There were few reports of antibacterial activity of acidic water from hot springs available in the world literature, mostly from Japan[5]. Hence such comparative study using alkaline hot spring water on Candida had got no support against which our results might be tallied. The results of the present study might suggest that the type and amount of non-living components (as the sample water was rendered microorganism free by autoclaving) were very important for the fungicidal activity on those very two strains of C. albicans. In summer and winter they showed better efficacy which may be attributed to dilution and decrease in pH of hot spring water by rain water. All the parameters tested here had shown encouraging results confirming that among those two sources, alkaline hot spring water from Taptapani was more effective or had naturally got more powerful activity. The findings led to the query that, was combination of Na^{+} and $SO^{=}_{4}$ effective for restricting the growth? A common belief prevailed that

$SO^{=}_4$ was effective as a healing agent. But in our study, effectiveness of the water of Bakreswar as healer was found less, though it contained more $SO^{=}_4$.This raised a question whether $SO^{=}_4$ should be administered strictly in an optimum dose for maximum effectiveness. Hence, finding out the right dose for administration of $SO^{=}_4$ as a local application should be taken up for further investigation and is very much likely to open up new avenues in field of dermatology regarding effective use of $SO^{=}_4$.

7. Conclusion

We wanted to infer that hot spring water exhibited inhibitory actions on growth and pathogenic effects of C. albicans. As in this study any bacteria / fungus in the sample water got sterilized, this result might totally represent the importance of some ions present in the hot-spring water, not an organic factor. However, further studies seemed warranted to ascertain probable clinical utilities.

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