

# A COMPARATIVE STUDY OF AQUEOUS EXTRACT OF THE GALLS OF QUERCUS INFECTORIA AND LEAVES OF CASSIA ALATA AGAINST CANDIDA ALBICANS IN VITRO

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**Key words:** antifungal activity, *Candida albicans*, *Cassia alata*, *Quercus infectoria* galls

## Introduction

Over three quarter of women have at least one episode of vaginal candidiasis. A few women have frequent recurrences (Monga, A, 2006, p 167). The typical presentation is with itching and soreness of the vagina and vulva, with a curdy, white discharge, which smell yeast, but in some cases there may be itching and redness with thin, watery discharge.

In Ayurvedic texts, various conditions giving rise to white discharge per vaginum have been described. *Śārangadharasamhitā*, *Bhāvaprakāśa*, *Yogaratnākara* and commentator *Cakrapāṇi* have used the word "Svetapradara" (Leucorrhoea) for white vaginal discharges. Leucorrhoea is not a disease, but a symptom of so many diseases and the term is loosely used for any type of whitish discharge per vaginum, which are mostly pathological arising from inflammatory

conditions of the genital tract (Joshi, N.G, 1999, p.112).

In the recent years, research on medicinal plants has attracted a lot of attention globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternative systems of treatment of human diseases. (Timothy S.Y and others, 2012) The development of resistance to most antimicrobial agents and the high cost of treatments have necessitated the search for new, safe, efficient and cost effective ways for the management of infectious conditions.

According to traditional medicine system in Sri Lanka and Ayurveda, Galls of *Quercus infectoria* and *Cassia alata* are very effective herbal materials which have been using against different kinds of fungal

infections. It can be used alone or can be used combined with other drugs and effective in systemic treatment as well as local application. There are some research publications about antimicrobial effect of galls of *Quercusinfectoria* and *Cassia alata*.

### Materials and Methods:

#### Plant Materials

The galls of *Q. infectoria* were purchased from the *GampahaSiddhayurveda Pharmaceuticals Pvt. Ltd.* and Leaves of *Cassia alata* were taken from the garden of *GampahaWickramarachchi Ayurveda Institute, University of Kelaniya*. They were authenticated by the botanists and a voucher specimen of *Cassia alata* plant was deposited for future reference.

#### Preparation of aqueous extract

For the preparation of aqueous extract, it was used the standard Ayurvedic method called *kvāthaparibhāsha*. (Nagodavithana, P., 2001) First the galls of *Quercusinfectoria* and leaves of *Cassia alata* were washed with distilled water. Then 60 grams of above materials were put into 02 separate containers and poured 1920 ml of distilled water and boiled it until reduce into 240 ml (Sample A - 1/8) using a heating plate. Then another two samples (Sample B - 1920ml reduced into 120ml [0.5/8] and Sample C - 1920 ml reduced into 60ml [0.25/8]) were made by using the first sample (Sample A) to determine the efficacy depending on the concentration.

#### Microorganisms

The fungus which was used in this study was *Candida albicans*, taken from Medical Research Institute, Colombo,

Sri Lanka, which was grown and maintained on SabouraudDextrose agar slants.

#### Preparation of inoculums

Inoculums were prepared by picked five distinct colonies from 24 hours old culture grown on SabouraudDextrose Agar which were incubated at 37 °C. Colonies were suspended in 10ml distilled water and vortex the resulting suspension and adjusted the turbidity to  $1 \times 10^5$  -  $1 \times 10^6$  cells/ml, using sterilized distilled water.

#### Preparation of culture media

65.0g of Sabouraud Dextrose Agar (Himedia Laboratories pvt. Ltd, Mumbai, Lot No. 00000040909, Jul 2013) was mixed in 1000ml distilled water and boiled until completely dissolved. Then it was sterilized by autoclaving under a pressure of 15 lbs/inch at 121 °C for 15 minutes. Culture media was prepared by pouring the mixture under aseptic conditions on to the sterilized petri dishes (diameter - 9 cm) in the same thickness of each.

#### Spreading the inoculums

0.1ml (100  $\mu$ l) of broth culture was spread evenly by using spreader under aseptic conditions.

#### Screening of antifungal activity

The well diffusion method was used to assess the anti-fungal effect. Anti-fungal activity was interpreted from the size of inhibition zones diameter which were measured to the nearest mm from observation of clear zones surrounding the wells. In case, 18 different holes were made on the media plate in which 50  $\mu$ l of the plant extracts and each

extract was assayed in triplicate in order to calculate the mean value. Sterile distilled water 0.05ml (50 $\mu$ l in a well) was served as negative control. A serial solution of Fluconazole (Fluconazole USP 50mg, Square Pharmaceuticals Ltd., Bangladesh,

Flugal® 50, D.A.R. No.321-28-62) was prepared in sterile distilled water and adjusted to a final concentration of 2.5 mg ml<sup>-1</sup> (125  $\mu$ g/50  $\mu$ l in a well) and used as the standard to confirm that the fungus was inhibited by the antifungal drugs (as a positive control)

## Results and Discussion

Table 01. - Paired sample test CA-QI

		Paired Differences				t	df	Sig. (2-tailed)	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower				Upper
Pair 1	CA_A-QI_A	-19.33333	1.15470	.66667	-22.20177	-16.46490	-29.000	2	.001
Pair 2	CA_B-QI_B	-19.33333	1.15470	.66667	-22.20177	-16.46490	-29.000	2	.001
Pair 3	CA_C-QI_C	-22.33333	1.15470	.66667	-25.20177	-19.46490	-33.500	2	.001

CA - *Cassia alata* QI - *Quercus infectoria* A - Sample A - 1920ml reduced into 240ml [1.0/8]

B - Sample B - 1920ml reduced into 120ml [0.5/8] C - Sample C - 1920 ml reduced into 60ml [0.25/8]

The effect of the water extract of the leaves of *Cassia alata* on *Candida albicans* at any concentration has shown a statistically significant difference ( $p < 0.05$ ) with the galls of the *Quercus infectoria*. (Table 01)

Positive control showed 25.67 mm, 27.00 mm and 24.67 mm as Mean Inhibitory Zone diameter in sample A, B and C respectively. Mean Inhibitory Zone diameter of all samples of positive control was 25.66 mm. Distilled water which was used as negative control has shown no response in all samples.

Table 02. - Paired sample test PC - QI

Paired Samples Test									
	Paired Differences					t	df	Sig. (2-tailed)	
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
				Lower	Upper				
Pair 1	QI_A-PC_A	-6.33333	.57735	.33333	-7.76755	-4.89912	-19.000	2	.003
Pair 2	QI_B-PC_B	-7.66667	.57735	.33333	-9.10088	-6.23245	-23.000	2	.002
Pair 3	QI_C-PC_C	-2.33333	1.52753	.88192	-6.12792	1.48125	-2.646	2	.118

**QI - *Quercusinfectoria* PC - Positive Control**

**A - Sample A - 1920ml reduced into 240ml [1.0/8] B - Sample B - 1920ml reduced into 120ml [0.5/8]**

**C - Sample C - 1920 ml reduced into 60ml [0.25/8]**

The water extract of the galls of *Quercusinfectoria* of sample A and B have shown a statistical significant difference ( $p < 0.05$ ) with the positive control (Fluconazole USP

125 $\mu$ g/50 $\mu$ l). But in the sample C (1920 ml reduced into 60ml [0.25/8]), it has not shown a significant difference with the positive control. (Table 02)

Paired Samples Test

Paired Samples Test									
	Paired Differences					t	df	Sig. (2-tailed)	
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference					
				Lower	Upper				
Pair 1	CA_A-PC_A	-25.66667	.57735	.33333	-27.10088	-24.23245	-77.000	2	.000
Pair 2	CA_B-PC_B	-27.00000	1.00000	.57735	-29.48414	-24.51586	-46.765	2	.000
Pair 3	CA_C-PC_C	-24.66667	.57735	.33333	-26.10088	-23.23245	-74.000	2	.000

**CA - *Cassia alata* PC - Positive Control**

**A - Sample A - 1920ml reduced into 240ml [1.0/8] B - Sample B - 1920ml reduced into 120ml [0.5/8]**

**C - Sample C - 1920 ml reduced into 60ml [0.25/8]**

Table 03. - Paired sample test PC - CA

The effect of the water extract of the leaves of *Cassia alata* on *Candida albicans* at any concentration has shown a statistically significant difference ( $p < 0.05$ ) with the positive control. (Table 03)

Galls of *Quercusinfectoria* and leaves of *Cassia alata* have been proven for anti-fungal activity including *Candida albicans* in many previous studies. Galls of *Quercusinfectoria* have been using successfully against clinically diagnosed vulvovaginal candidiasis in general practices in Ayurveda and leaves of *Cassia alata* also have been using against fungal infections. According to the previous studies though *Cassia alata* has been proven for its anti-fungal effect, in this study it gave no significant response. It can be assumed that the concentration of the samples were not enough to give a significant response at expected level against *Candida albicans*.

### Conclusion

From the above results and discussion it can be concluded that the aqueous extract of galls of *Quercusinfectoria* has shown anti-fungal effect against *Candida albicans* without a significant difference ( $P > 0.05$ ) while comparing with the tested concentration of Fluconazole USP ( $125 \mu\text{g}/50 \mu\text{l}$ ).

The aqueous extract of the leaves of *Cassia alata* did not give any significant response against *Candida albicans* at the tested concentrations.

**Acknowledgement:** The authors like to convey their gratitude to Dr. D.L. Jayarathne, senior lecturer, University of Kelaniya and to Dr. W.M.B. Weerasooriya, senior lecturer,

Gampaha Wickramarachchi Ayurveda Institute for their support in the completion of this work.

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