



Research Article

ANTIFUNGAL ACTIVITY OF *PANCHAWALKALA KWATHA* AND *PANCHAWALKALA SHATADAUTA GHRITA*

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ABSTRACT

Panchawalkala is one of the ideal herbal combinations in Ayurveda and it has therapeutic properties such as *Vranaropana*, *Shothahara*, *Graahi* and *Visarpahara*. And also researchers have been proven anthelmintic, antimicrobial, wound healing and anti-inflammatory activities of these plants in combination and individual too. *Ghrita* (Ghee) is one of the oil preparation made by cow's milk and it has *Balavardhaka*, *Ojovardhaka*, *Vayasthapaka*, *Dhatuposhaka* properties and is supreme in *Snehana Dravyas*. *Panchawalkala* is widely used in *Kwatha* and Powder form. *Shatadauta Ghrita* is the most famous form of the *Ghee*. The advantages of different innovative preparations are; increased shelf life, ready to use, better acceptability and ease of application. This study was planned to evaluate in-vitro antifungal activity of traditional *Panchawalkala Kwatha* and innovative *Panchawalkala Shatadauta Ghrita* (the *Ghrita*, hundreds times purified by *Panchawalkala Kwatha*). It was assessed by adopting agar diffusion method. Each agar plate was divided into four equal parts and was cultivated the *Candida albicans*. Replicator device was used to inoculate multiple specimens on to two parts of three series of plates with respective drugs. Further, responses of organism to the trial drugs were measured and compared with standard drug of Fluconazole (+ve control) and distilled water (-ve control) by using other two parts of the agar plates. All the plates were incubated at 37°C for 24 hours. According to the findings, *Panchawalkala Shatadauta Ghrita* has an antifungal effect than *Panchawalkala Kwatha*. Fluconazole is the best antifungal drug among these and distilled water does not have any antifungal action. Hence, it can be concluded that, *Panchawalkala Shatadauta Ghrita* has antifungal activity rather than *Panchawalkala Kwatha* but not effective than Fluconazole.

KEYWORDS: *Candida albicans*, *Panchawalkala Kwatha*, *Panchawalkala Shatadauta Ghrita*, Antifungal activity.

INTRODUCTION

Panchawalkala is the great combination which was mentioned under *Nyagrodhadighana*^[1] in ancient Ayurvedic classics. According to that, *Nyagrodha* (*Ficus benghalensis* Linn.), *Udumbara* (*Ficus glomerata* Roxb.), *Ashvatha* (*Ficus religiosa* Linn.), *Parisha* (*Thespesia populnea* Soland ex correa) and *Plaksha* (*Ficus lacor* Buch-Ham.) are the prescribed barks of the *Panchawalkala*. In *Raja Nighantu*, following plants are prescribed as *Panchawalkala*. These are *Nyagrodha*, *Udumbara*, *Ashvatha*, *Plaksha* and *Amlawetasa* (*Garcinia pedunculata* Roxb.). However, most scientific

research has been proven both recipes have same properties. And also, these classics have been described medicinal properties of *Panchawalkala*^[2] as follows: *Vranaropana*, *Shothahara*, *Graahi* and *Visarpahara*. Researchers have been proven antiseptic, anti-inflammatory, immune-modulatory, antioxidant, antibacterial, antimicrobial, wound purifying and wound healing effect of these plants in combination and individual too. Furthermore; *Panchawalkala* is an ideal drug for the treatment of Hemorrhoids, *Kushta Roga* (skin diseases), and *Vrana Roga*.

Figure 1: Ingredients of Panchawalkala

<p>Sanskrit Name: <i>Nyagrodha</i> Botanical Name: <i>Ficus benghalensis</i> Linn. Common Name: Banyan Tree</p>			
<p>Sanskrit Name: <i>Udumbara</i> Botanical Name: <i>Ficus glomerata</i> Roxb. <i>(Ficus racemosa</i> Linn.) Common Name: Cluster fig</p>			
<p>Sanskrit Name: <i>Ashvatha</i> Botanical Name: <i>Ficus religiosa</i> Linn. Common Name: Sacred fig</p>			
<p>Sanskrit Name: <i>Parisha</i> Botanical Name: <i>Thespesia populnea</i> Linn. <i>(Soland. ex correa)</i> Common Name: Portia Tree</p>			
<p>Sanskrit Name: <i>Plaksha</i> Botanical Name: <i>Ficus lacor</i> Buch-Ham. Common Name: Java fig</p>			
<p>Sanskrit Name: <i>Amlawetasa</i> Botanical Name: <i>Garcinia pedunculata</i> Roxb. Common name: Bor Thekera</p>			

Ghee known as *Ghritam*, *Havish*, *Sarpish* and *Ajya*, was used in ancient India as early as 1500 B.C. The *Rigveda* (the oldest collection of Hindu hymns) contains numerous references on *ghee*, showing its importance in Indian diet. The health benefits of *Ghee* can be obtained from consuming *ghee* as food and using *ghee* as a medicine. Clarified milk fat or butter fat is known as *Ghrita* and it is prepared by heating butter or cream to remove water content. The *Go-Ghrita* is the best of choice for food and medicinal purposes. *Ghrita* is one among the best *Ajasrika Rasayanas*. And also, it has *Balavardhaka*, *Ojovardhaka*, *Vayasthapaka*, *Dhatuposhaka* and is supreme in *Snehana Dravyas*.^[3]

The *Shatadauta Ghrita* is the *Ghrita*, hundreds times purified by the water. *Panchawalkala Shatadauta Ghrita* is an innovative drug and it was prepared by hundreds times purifying the *Ghrita* using the *Panchawalkala Kwatha* instead of the water.

Figure 2: Panchawalkala Shatadauta Ghrita



Figure 3: Panchawalkala Kwatha



The laboratory findings (in-vitro study) are the one of most effective scientific way to prove the effectiveness of a drug. Therefore this study was designed. Previous research was already proven the antifungal action of Fluconazole, *Panchawalkala Kwatha* and *Shatadauta Ghrita*. However, there was no research conducted for *Panchawalkala Shatadauta Ghrita* because of it is an innovative drug.

This study was designed to evaluate in-vitro antifungal activity of *Panchawalkala Kwatha* and *Panchawalkala Shatadauta Ghrita*.

MATERIALS AND METHODS

Preparation of the Panchawalkala Kwatha

The *Panchawalkala kwatha* made from one part of herbs (*Nyagrodha*, *Udumbara*, *Ashvatha*, *Parisha* and *Plaksha*) in sixteen parts of water, which is reduced to 1/8th part of liquid after cooking on a low flame.^[4] Then after, it was filtered by using the piece of cloth and store in a clear glass bottle.

Table 1: Composition of the Panchawalkala ^[5]

Drug	Latin Name	Common Name	Part used	Proportion
<i>Nyagrodha</i>	<i>Ficus benghalensis</i> Linn.	Banyan Tree	Bark	1 part
<i>Udumbara</i>	<i>Ficus glomerata</i> Roxb.	Cluster fig	Bark	1 part
<i>Ashvatha</i>	<i>Ficus religiosa</i> Linn.	Sacred fig	Bark	1 part
<i>Parisha</i>	<i>Thespesia populnea</i> Linn. (<i>Soland. ex correa</i>)	Portia Tree	Bark	1 part
<i>Plaksha</i>	<i>Ficus lacor</i> Buch-Ham.	Java fig	Bark	1 part

Preparation of Panchawalkala Shatadauta Ghrita

Ghrita was purified by using prepared *Panchawalkala Kwatha* and filtered. This procedure was repeated for hundreds times. Then, it was called as *Panchawalkala Shatadauta Ghrita*.

Antimicrobial activity

Micro-organisms are broadly classified as Bacteria, Fungi, Viruses and Actinomytes and they are causative factors in the manifestation of various infectious diseases. Hence, detailed study of these micro-organisms, the ways in which they produce diseases in human body and information regarding diagnosis and treatment is essential.

In-vitro study are used as screening tests for new agents and for testing the susceptibility of individual isolates from infections to determine

which of the respective drug might useful therapeutically.

In general, any compound or drugs that inhibit the growth or cause the death of micro-organisms are known as antimicrobial agents or drugs and any drug that inhibits the growth of fungi is called as fungistatic activity. The present study was planned to check comparative antifungal activity of the *Panchawalkala Kwatha* and *Panchawalkala Shatadauta Ghrita*.

Candida albicans was used as a micro-organism for this study.

Place of Study

Preparation of *Panchawalkala Kwatha* and *Panchawalkala Shatadauta Ghrita* has been

performed at the *Bhaishajyagara* (Ayurveda Pharmacy) of the Faculty of Indigenous Medical Sciences in the Gampaha Wickramarachchi University of Indigenous Medicine, Yakkala, Sri Lanka.

The antifungal study has been performed at Laboratory of the Gampaha Wickramarachchi University of Indigenous Medicine, Yakkala, Sri Lanka.

Antifungal Sensitivity Test

Kirby-Bauer test (disk diffusion test/disk diffusion antibiotic sensitivity test) was adopted for this study. In this method culture based microbiology assay used in diagnostic and drug discovery laboratories.^[6]

Material Requirements

- Nutrient Agar (for bacterial cultivation)
- 2-15 hours (overnight) young culture of *Candida albicans*
- Standard solution antibiotics
- Different concentrations of trial drugs
- Sterile petri dishes and sterile cork-borer
- Incubator and laminar air flow cabinet

Preparation of the Agar medium

16.2g of Sabouraud's Dextrose Agar 16.2g was dissolved in 250ml of distilled water and then after, autoclaved at 121°C for 15 minutes for sterilization.

Preparation of the nutrient broth

3.5g of commercial available nutrient medium was dissolved in 250ml of distilled water and was dispensed as desired and sterilized by autoclaving at 15lbs pressure at 121°C for 15 minutes. The broth was kept to cool in room temperature and *Candida albicans* fungus incubates in to grow at 37°C for 18 hrs.

RESULTS AND DISCUSSION

Preparation of peptone water

0.75g of peptone powder was suspended in 50ml of distilled water and it was dispensed as desired and sterilized by autoclaving at 15lbs pressure at 121°C for 15 minutes.

Inoculum Preparation

Loopful of standard strain of *Candida albicans* was suspended in 50ml of nutrient broth. 1ml of nutrient broth was suspended in 9ml of peptone water.

Preparation of Media plates

20ml of autoclaved agar medium was poured into sterile glass flat-bottom three petri dishes (90mm diameters) with uniform depth of 4mm and was kept room temperature for solidify.

Cultivation of *Candida albicans*

100µl of dilution series was got into pipette and spread on the agar medium (petri dishes) by using sterile cotton swab. This procedure was repeated two or more times.

Sample preparation and testing

Prepared three cultivated agar petri dishes were divided into equal four sections and they were filled with 50µl of *Panchawalkala Shatadauta Ghrita* (named as Ps), 50µl of *Panchawalkala Kwatha* (named as P), 50µl of distilled water (named as negative control) and 50µl of Fluconazole (named as positive control). All the plates were incubated at 37°C for 24 hours.

Data collection and analysis

Diameter of the inhibitory zones was reported in Millimeter (mm) by using foot ruler. Collected data was analyzed by using SPSS statistical software.

Figure 4: Results of antifungal tests

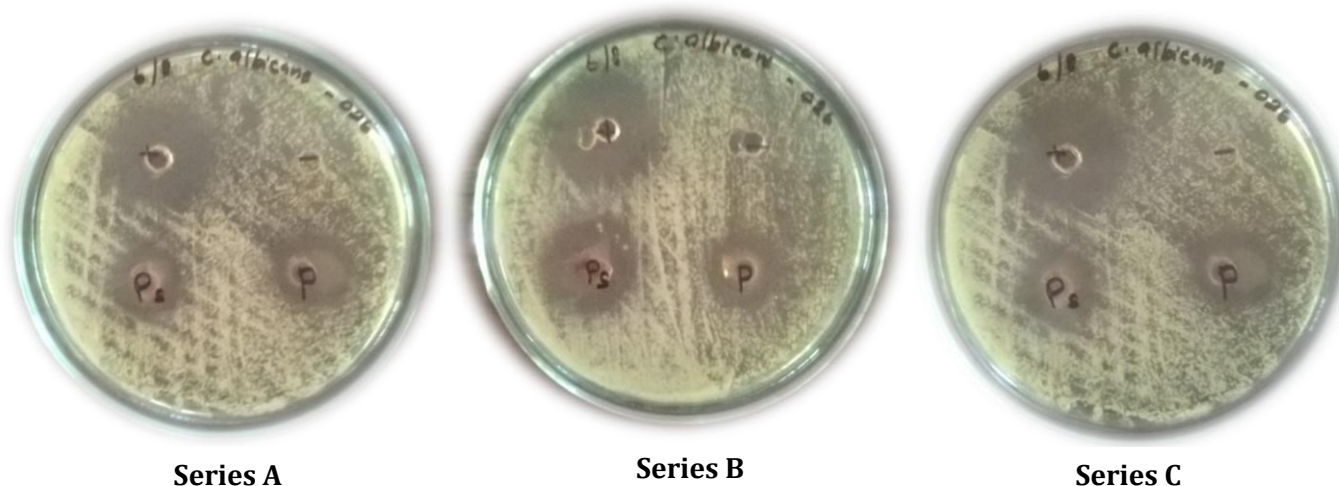


Table 2: Results of antifungal test

Testing Sample	Inhibition Zone Diameter (mm)			
	Series A	Series B	Series C	Mean
Positive (+) Control	28	30	29	29
Negative (-) Control	0	0	0	0
<i>Shatapanchawalkaladauta Ghrita</i>	19	20	18	19
<i>Panchawalkala Kwatha</i>	16	14	15	15

Table 3: Data analysis results (ANOVA test)

Inhibition zone diameter

	Sum of squares	df	Mean square	F	Sig.
Between groups	312.000	2	156.000	156.000	.000
Within groups	6.000	6	1.000		
Total	318.000	8			

Table 4: Multiple Comparison results (Post Hoc test)

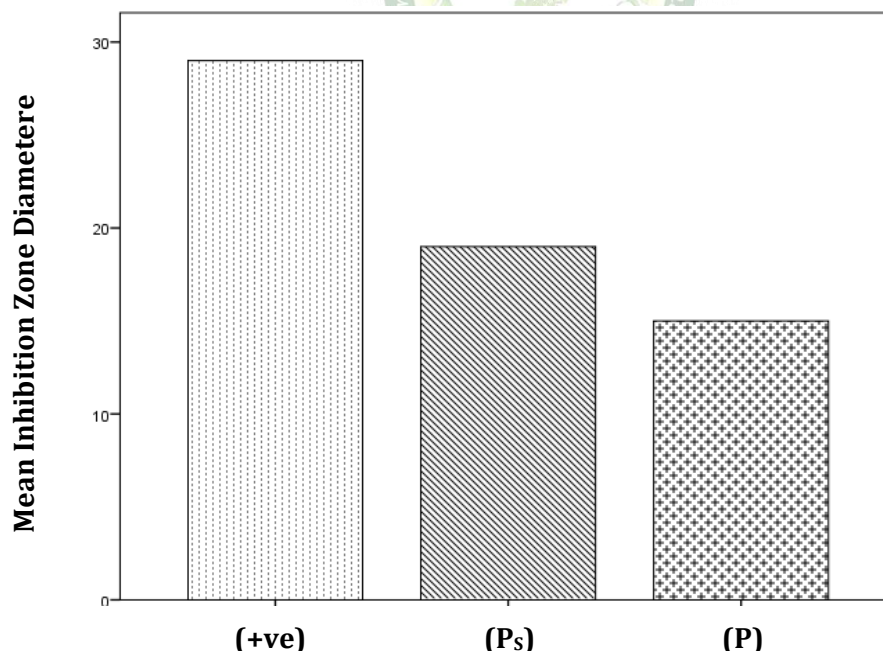
Dependent variable: Inhibition zone diameter

Turkey HSD

(I) Drug	(J) Drug	Mean Difference (I-J)	Std. error	Sig.	95% confidence Interval	
					Lower bound	Upper bound
Positive control (+ve)	(Ps)	10.000*	.816	.000	7.49	12.51
	(P)	14.000*	.816	.000	11.49	16.51
<i>Shatapanchawalkaladauta Ghrita</i> (Ps)	(+ve)	-10.000*	.816	.000	-12.51	-7.49
	(P)	4.000*	.816	.006	1.49	6.51
<i>Panchawalkala Kwatha</i> (P)	(+ve)	-14.000*	.816	.000	-16.51	-11.49
	(Ps)	-4.000*	.816	.006	-6.51	-1.49

The mean difference is significant at the 0.05 level

Figure 5: Mean volume chart



DISCUSSION

These findings show the beneficial effects of selected herbal formulation for *Candida albicans*. According to the data analysis, the mean difference is significant at the 0.05 level. (+ve) control and *Panchawalkala Shatadauta Ghrita* resulted significant reduction of P<0.00 (P<0.05). And also (+ve) control and *Panchawalkala Kwatha* resulted significant

reduction of P<0.00 (P<0.05). *Panchawalkala Shatadauta Ghrita* and *Panchawalkala Kwatha* resulted significant reduction of P<0.006 (P<0.05). Therefore, it is very clear that the *Panchawalkala Shatadauta Ghrita* has an antifungal action against the *Candida albicans*. Furthermore, the results show that the *Panchawalkala Shatadauta Ghrita* has more

inhibitory effect than the *Panchawalkala Kwatha*. This tends to show that the active ingredient of the bark of the *Panchawalkala* is a better extracted with adding *Ghee (Ghrita)* than water. However, the *Panchawalkala Shatadauta Ghrita* has no effect than Fluconazole. Although, the *Panchawalkala Shatadauta Ghrita* have no side effects as Fluconazole.^[7]

Antibacterial action of *Panchawalkala* powder and ointment has been proven by researchers.^[8] Wound healing property (*Vranaropana*) of *Panchawalkala* has been scientifically proven by various research.^[9-11]

CONCLUSION

According to the findings, *Panchawalkala Shatadauta Ghrita* has an antifungal action rather than *Panchawalkala Kwatha*. Fluconazole is the best antifungal drug among these and distilled water has not antifungal action. Hence, it can be concluded that, *Panchawalkala Shatadauta Ghrita* is better than *Panchawalkala Kwatha* but not better than Fluconazole in case of *Candida albicans*.

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