Research Article

ANTIFUNGAL ACTIVITY OF PANCHAWALKALA KWATHA AND PANCHAWALKALA SHATADUTA GHrita

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ABSTRACT

Panchawalkala is one of the ideal herbal combinations in Ayurveda and it has therapeutic properties such as Vrnanopana, Shothahara, Graahi and Visarpahara. And also researchers have been proven anthelmitic, 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. Shataduta 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 Shataduta 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 Shataduta 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 Shataduta Ghrita has antifungal activity rather than Panchawalkala Kwatha but not effective than Fluconazole.

KEYWORDS: Candida albicans, Panchawalkala Kwatha, Panchawalkala Shataduta Ghrita, Antifungal activity.

INTRODUCTION

Panchawalkala is the great combination which was mentioned under Nyagrodhagandha[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

| Sanskrit Name | Botanical Name | Common Name |
|---------------|----------------|-------------|
| Nyagrodha     | *Ficus benghalensis* Linn. | Banyan Tree |
| Udumbara      | *Ficus glomerata* Roxb. | Cluster fig |
| Ashvatha      | *Ficus religiosa* Linn. | Sacred fig |
| Parisha       | *Theespesia populnea* Linn. | Portia Tree |
| Plaksha       | *Ficus lacor* Buch-Ham. | Java fig |
| Amlawetasa    | *Garcinia pedunculata* Roxb. | Bor Thekera |

Ghee known as Ghritam, Havish, Sarpih 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. Then after, it was filtered by using the piece of cloth and store in a clear glass bottle.

| Drug       | Latin Name                     | Common Name   | Part used | Proportion |
|------------|--------------------------------|---------------|-----------|------------|
| Nyagrodha  | Ficus benghalensis Linn.       | Banyan Tree   | Bark      | 1 part     |
| Udumbara   | Ficus glomerata Roxb.          | Cluster fig   | Bark      | 1 part     |
| Ashvatha   | Ficus religiosa Linn.          | Sacred fig    | Bark      | 1 part     |
| Parisha    | Thespesia populnea Linn. (Soland. ex correa) | Portia Tree | Bark | 1 part |
| Plaksha    | Ficus lacor 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 available online at: http://ijapr.in
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 P), 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](image-url)
Table 2: Results of antifungal test

| Testing Sample               | Series A | Series B | Series C | Mean |
|------------------------------|----------|----------|----------|------|
| Positive (+) Control         | 28       | 30       | 29       | 29   |
| Negative (-) Control         | 0        | 0        | 0        | 0    |
| Shatapanchavalkaladauta Ghrita | 19       | 20       | 18       | 19   |
| Panchawalkala Kwatha         | 16       | 14       | 15       | 15   |

Table 3: Data analysis results (ANOVA test)

|                      | 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)

| (I) Drug                           | (J) Drug   | Mean Difference (I-J) | Std. error | Sig.   | 95% confidence Interval | Figure 5: Mean volume chart |
|------------------------------------|------------|-----------------------|------------|--------|-------------------------|-----------------------------|
| Positive control (+ve)             | (P_3)      | 10.000*               | .816       | .000   | 7.49-12.51              | +ve                         |
| (P)                                |            | 14.000*               | .816       | .000   | 11.49-16.51             |                            |
| Shatapanchavalkaladauta Ghrita (P_3) | (+ve)     | -10.000*              | .816       | .000   | -12.51-7.49             | (P_3)                       |
| (P)                                |            | 4.000*                | .816       | .006   | 1.49-6.51               |                            |
| Panchawalkala Kwatha (P)           | (+ve)      | -14.000*              | .816       | .000   | -16.51-11.49            | (P)                         |
| (P_3)                              |            | -4.000*               | .816       | .006   | -6.51-1.49              |                            |

The mean difference is significant at the 0.05 level

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). 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 Shataduta Ghrita has no effect than Fluconazole. Although, the Panchawalkala Shataduta 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 Shataduta 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 Shataduta Ghrita is better than Panchawalkala Kwatha but not better than Fluconazole in case of Candida albicans.

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