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EFFICACY OF ALCOHOLIC EXTRACT OF DHATAKI (*WOODFORDIA FRUTICOSA*) AGAINST *CANDIDA ALBICANS* FROM *KAPHAJA YONIVYAPAT* (VULVO VAGINAL CANDIDIASIS)

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ABSTRACT

Women represent the cornerstone of a family's overall health, ensuring they have access to quality care leads to improvement in health of children as well as the whole family. Vulvo vaginal candidiasis is the second most common infection among reproductive aged women with a single incidence of 75% and two or more episodes of 45% of women. Clinical features of vulvo vaginal candidiasis finds similarity with features of Kaphaja yonivyapad mentioned in ayurvedic classics. The drug Dhataki is attributed with Krimigna action in ayurvedic literature and studies have shown its antimicrobial action against various organisms. In the current study,action of alcoholic extract of Dhataki against Candida albicans is evaluated from vaginal swab sample collected from patients diagnosed with kaphaja yonivyapad(Vulvo vaginal candidiasis) by culture and sensitivity. With the current study, it is evident that the mean zone of inhibition of alcoholic extract of Dhataki possesses anti-microbial action against the fungus Candida albicans. Further, it is also obvious that as the concentration of aqueous extract of Dhataki increases, the zone of inhibition also increases.

KEYWORDS: *Vulvo vaginal candidiasis,Kaphaja yonivyapad,Candida albicans,Culture and sensitivity,Dhataki,Alcohol extract*

INTRODUCTION

Vulvovaginitis or inflammation of the vulva and vagina most commonly occurs in reproductive aged women and is usually secondary to infection.Candidal vulvovaginitis is responsible for about one third of cases[1].Candidal vulvovaginitis is caused by inflammatory changes in the vaginal and vulvar epithelium secondary to infection with candida species most commonly candida albicans.Candida is part of the normal flora in many women and is asymptomatic.Therefore candida vulvovaginitis requires both the presence of candida in the vagina as well as the symptoms of irritation,itching,dysuria or inflammation.

Vaginal swab culture and sensitivity is identified as a tool to identify such organism responsible for the infection and through sensitivity evaluation appropriate drug is selected for the management. Even though many drugs are attributed with krimighna action in Ayurveda, there are only a few works done on establishing the effectiveness of specific drug activity on specific micro-organisms. Hence such drugs need to be analysed for action against specific micro-organisms so that an upashaya effect of such drugs can be generated on micro-organisms invitro. *Dhataki* possesses *krimigna* action according to ayurvedic classes Taking over new methods like culture and sensitivity will strengthen existing ayurvedic knowledge for achieving improved diagnostic and curative abilities. Hence the present study is undertaken to review various attributes of the fungus *Candida albicans*, its laboratory diagnosis, culture, and evaluate *Upashaya* capability invitro by the sensitivity with extract of plant *Dhataki*.

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Dhataki though ascribed with *Krimighna* action its efficacy on *Candida albicans* from vaginal swab sample has not been investigated. Hence in present work, alcoholic extract of *Dhataki(Woodfordia fruticosa)* for culture and sensitivity against *Candida* from vaginal sample of the subject are to be evaluated.

AIMS AND OBJECTIVES

To evaluate the sensitivity of alcoholic extract of *Dhataki(Woodfordia fruticosa)*against *Candida albicans* from vaginal sample of *Kaphaja yonivyapad* (Vulvovaginal candidiasis) patients by culture and sensitivity in vitro.

MATERIALS AND METHODS

A minimum of 30 subjects aged between 18-55 years irrespective of caste and religion,diagnosed with candidiasis presenting with following lakshanas- *Picchila yoni srava* (slimy thick vaginal discharge), *Kandu* (vaginal pruritis) with or without symptoms of *Alpavedana* (dull pain), *Panduta* (paleness) and *Pandu picchila arthava* [2](pale mucoid menstrual blood) from out-patients and in-patients departments of tertiary ayurvedic hospital, Hassan was included in the study. Microbial infections other than *candida albicans,*Pregnant and lactating women and omen on their menstrual phase were excluded from the study

RESEARCH DESIGN

An observational experimental study

METHODOLOGY

Alcoholic extract of Dhataki was prepared using cold maceration[3] method using 50g each of fresh and clean *Dhataki* weighed using a weighing balance.The flowers were then crushed to powder in a clean mortar and pestle finely without adding water.Powdered 50gm was added to 250 ml ethanol taken in a 1000ml capacity conical flask .The conical flask was then plugged tightly with cotton and was sealed with tape.The conical flask was shaken manually for 10-15 minutes at an interval of every 3 hours during the daytime.The procedure was repeated for seven days. On the 7th day, the content of the conical flask was filtered, which yielded 210 ml of alcoholic filtrate .The filtrate was then kept over a water bath in a China dish at 60^0C.5 gram of alcoholic extract of *Dhataki* was obtained by this process.

Vaginal samples were collected from patients using sterile cotton swab.A portion of sample was used for direct microscopic characterization of fungi and from the remaining a loop full of inoculum was used for culture by streaking on Potato dextrose agar plates and was placed in incubator at 370C and cultured for 24-48 hours. After 24-48 hours of incubation, the cultural characteristics like colony morphology were studied and microscopic observation was done

The results that showed positive cultures for *Candida albicans* was further examined for sensitivity with Dhataki.Sensitivity test was done using Agar well diffusion method. Cleaned the workplace in laminar airflow using 70% ethyl alcohol and switched on to UV for 20 minutes.Poured around 15 ml Potato Dextrose Agar media uniformly over the Petri dish, mixed well, and allowed the media to solidify for 30 minutes.One loop full of *Candida albicans* from 24-48 hours culture was transformed into the Potato Dextrose Agar plate (one for each extract) with a sterile, non- toxic cotton swab and swabbed over the media (lawn culture).Made six equidistant wells on both the plates with a sterile cork borer and added different concentrations of alcoholic extract into wells on the other plate.Tests were conducted for five different concentrations of aqueous and alcoholic extracts of *Dhataki*(3000µg/ml,2000 µg/ml,2000 µg/ml,1000 µg/ml 500 µg/ml,100µg/ml) and with control of ethanol.Incubated the petri plates at 370C for 24-48 hours. After the incubation period the zone of inhibition were measured in mm with a ruler.

ASSESSMENT CRITERIA

Initially 6 wells in each plate were charged with 6 different concentrations of alcohol extract of*Dhataki*. If the drug is sensitive a clear circular “halo” (known as zone of inhibition) appears around the well, indicating absence of fungi. If that zone appears, it shows that the particular drug is effective against *Candida albicans* fungi.

Analytical parameters

- Sensitive (S) zone
- Moderately sensitive (MS) zone
- Resistant (R) zone

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OBSERVATION AND RESULTS

Invitro anti- bacterial activity of alcoholic extract of *Dhataki* was evaluated by agar well diffusion method and zone of inhibition was measured as shown in Table

Table No. 1 : Mean values of zone of inhibition different concentrations ofalcoholic extract of *Candida albicans*

Different concentrations of alcoholic extract of*Candida albicans*	3000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml	100 µg/ml	Control
N	30	30	30	30	30	30
Mean	20.57	19.17	17.60	15.47	13.43	6.90

Table No. 29: Sensitivity test for alcoholic extract of different concentrations of Dhataki

Concent-rations	3000 µg/ml			2000 µg/ml			1000 µg/ml			500 µg/ml			100 µg/ml			control		
	S	MS	R	S	MS	R	S	MS	R	S	MS	R	S	MS	R	S	MS	R
No. of samples	20	8	2	18	8	4	15	11	4	9	11	10	6	9	15	0	3	27

The present study shows that the susceptibility of *Candida albicans* against the alcohol extract of *Dhataki* is fairly evident between 26mm to 22mm. Hence it is considered as sensitive. 18 to 14mm is considered as moderately sensitive. 12 to 8mm is considered as resistant. Therefore, with the current study it is evident that candida albicans organism is sensitive to 3000µl, 2000µl,1000µl; moderately sensitive to 500µl, whereas it is resistant to 100µl of alcoholic extract of *Dhataki.*

DISCUSSION

In the present study 40 subjects with *kaphaja yonivyapat* (vulvo vaginal candidiasis) were screened. Among them, 30 subjects fulfilled the diagnostic inclusion criteria, and the remaining 10 subjects were excluded.Among excluded samples were samples other than candida albicans and also samples of pregnant and lactating women. Plants and their constituents are the finest choice than any other synthetic chemical. Most of the formulations consist of plants and their phytochemical constituents as the chief component.In *Dhanwantari Nighantu*[4] and *Kaiyyadeva Nighantu*[5] *Dhataki* has been attributed with *Krimighna* action. In the present study, the cold maceration method was selected as it is easy to perform, economical and simple without using any complex instruments but yields a highly potent extract with several active principles. By assessing the mean values of the zone of inhibition shown by the alcoholic extract of *Dhataki* against *Candida albicans*, it was observed that the organism is sensitive to 3000µl and 2000µl and 1000µl; and moderately sensitive to 500µl; whereas it is resistant to 100µl.The phytochemical constituents present in the Dhataki alcoholic extract interferes with different mechanisms of candida, like altering the surface tension of the extracellular medium of organism cells, obstruding DNA of organism cells, complexing with extracellular and soluble proteins, etc. Different strains of fungus have anti-microbial effects including inhibition of various cellular processes followed by an increase in plasma membrane permeability and, finally, ion leakage from the cells[6] . Different concentrations of alcoholic extract of *Dhataki* showed different zones of inhibition. This is because different components diffuse at different rates that produce varying zones of inhibition against the fungus candida albicans. In higher concentrations of aqueous extract, the drug content is more, hence showing a significant zone of inhibition. On diluting the concentrations, the active constituents fully dissolve into the solution. So, the drug is incapable of giving antimicrobial action even though it reaches and is set at the cell membrane.

CONCLUSION

From this study, it is evident that the mean zone of inhibition of alcoholic extract of *Dhataki* (Woodfordia fruticosa) possesses antimicrobial action against candida albicans obtained from the vaginal sample of subjects diagnosed with *kaphaja yonivyapad* (Vulvo vaginal candidiasis). It is also evident that as the concentration of alcoholic extract of *Dhataki* (Woodfordia fruticosa) increases, the zone of inhibition for candida albicans also increases.

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