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Formulation and evaluation of antifungal activity of *Kasisadi Varti* against Candida albicans

Research Article

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Abstract

Background: Medicine is said to be of good quality when it gives the desired therapeutic effect and at the same time does not cause any harm to the patients. The safety and efficacy of a medicine is of at most importance. Preclinical studies play a vital role in ascertaining the safety and efficacy of a drug before taking it for clinical trial. They mainly involve invitro and invivo models. *Kasisadi churna* is a formulation mentioned for external application in *kaphaja yoni vyapath*. Application of *churna* (powder) externally may not be compatible on patients, and hence the dosage form was modified into *varti* (suppository) form. Objective: Invitro study to asses antifungal activity of *kasisadi varti.* Material and Methods: *Kasisadi varti* were prepared in three different methods. They were assessed for activity against *Candida albicans* by invitro Agar well diffusion method at different concentrations 32mg/dl, 64mg/dl, 128mg/dl, 256mg/dl and 512 mg/dl. Result: All three samples showed significant activity against *Candida albicans* at a concentration of 128 mg/dl, 256mg/dl and 512mg/dl. Conclusion:All three samples showed significant results on *Candida albicans* developed from vaginal smears of patients at a concentration of 128mg/ml, 256mg/ml, and 512 mg/ml in comparison with standard drug Amphotericin B. None of the samples showed any effect on MTCC Strain of *Candida albicans.*

Key Words: *Kasisadi varti,* Antifungal activity, *Candida albicans*, Invitro study.

Introduction

Medicine is said to be of good quality when it gives the desired therapeutic effect and at the same time does not cause any harm to the patients (1). The safety and efficacy of a medicine is of at most importance. Preclinical studies play a vital role in ascertaining the safety and efficacy of a drug before taking it for clinical trial. They mainly involve invitro and invivo models.

Kasisadi churna is a formulation mentioned for external application in *kaphaja yoni vyapath* along with honey(2). But the administration of the drug through vaginal route in this form may not be compatible to the patients. Modification of a dosage form will help in enhancement of efficacy, acceptability of the product and shelf life. When compared to *churna kalpana* (powder dosage form), *varti* (suppository) are having more shelf life and can be easily administered. *Kasisadi varti* consists of *kasisa, haritaki, amalaki, vibhitaki, sphatika, jambuasthi, amrasthi* and *dhataki* (2) (table 1). *Kaphaja yoni vyapath* is a clinical entity characterized by itching and mucoid discharge (3). It is correlated to vulvo vaginitis(4), which is usually caused by the organism *Candida albicans* (5,6). In the present study three samples of *kasisadi varti* were experimentally evaluated for their action against *Candida albicans* through in vitro method.

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Materials and methods

Pharmaceutical study (7)

The ingredients of *Kasisadi churna* are given in table 1.

Vati (tablet) is a solid dosage form of medication prepared by liquefying *guda* (jaggary), *sharkara* (sugar), *guggulu* and adding fine powders of *aushadha dravya* (medicinal drugs). It can also be prepared by pounding fine powder of *aushadha dravya* (medicinal drugs) with *guda* or *guggulu* or by triturating it with honey or any of the liquid preparation and then rolling into pill(17). If the *gutika* or *vati* (tablet) is modified into long oval shaped solid form or wick like shape, then it is called as *varti* (suppository). This is commonly used for local administration of following routes *guda* (anus), *yoni* (vagina), *shishna* (penis), *netra* (eye) and others. According to site of application and action they are categorized as *yoni varti*, *guda varti*, *netra varti*, *dhumra varti*, *nasa varti*, *vrana varti* and *shishna varti*. Based on the route of administration the length and diameter of the *varti* will vary(18).

Three samples of *varti* were prepared as mentioned below.

- Sample 1 - KV 1 – Prepared by *jala* (water) *bhavana* (trituration) method
- Sample 2 - KV2 – Prepared by *gudapaka* method
- Sample 3 - KV3 – Prepared by addition of cocoa butter as base

The experimental trials were done using culture of *Candida albicans* from different vaginal smears obtained from female patients on opd basis.

Table 1: Ingredient of *Kasisadi churna*

Drug	Botanical name	Part Used	Family/Kula	Chemical composition
***Kasisa*(8)**	-	Whole	-	Ferrous sulphate
***Haritaki*(9)**	*Terminalia chebula Retz.*	Fruit	Combretaceae *Haritaki Kula*	Tannin, Chebulic acid, Gallic acid, sorbitol,
***Vibhitaki*(10)**	*Terminalia bellerica Roxb.*	Fruit	Combretaceae *Haritaki Kula*	Tannin, gallic acid, ellagic acid, ethyl gallate, mannitol
***Amalaki*(11)**	*Emblica officinalis Gaertn.*	Fruit	Euphorbiaceae *Eranda Kula*	Ellagic acid, Amlaic acid, Phyllantine, Chebulic acid
***Sphatika*(12)**	-	Whole	-	Potassium AluminiumSulphate
***Jambu asthi*(13)**	*Syzigium cumini Skeels.*	Seed coat	Myrtaceae *Lavanga Kula*	Gallic acid, Citric acid, Glycollic acid, Ellagic acid, Corilagin
***Amra asthi*(14)**	*Mangifera indica Linn.*	Seed coat	Anacardiaceae *Amra Kula*	Mangiferin, Amino acids, Gallic acid, Vit C, Geraniol
***Dhataki*(15)**	*Woodfordia fruticosa Kurz.*	Flower	Lythraceae *Madayantika*	Becogenin, Gallic acid, ellagic acid, tannin, mesonositol
***Bhringaraja*(16)**	*Eclipta alba Hassk.*	Leaves	Asteraceae *Bhringaraja*	Phytosterol-A, Triterpenic acid, Linoleic acid

Experimental Study

Preparation of aqueous extract

25 gm of *kasisadi varti* (all the three samples) were made into powder and was placed inside a thimble made from thick filter paper which was loaded into the main chamber of the Soxhlet extractor. 150 ml of distilled water as the extraction solvent was taken into a distillation flask and the Soxhlet extractor was now placed onto this flask, and was then equipped with a condenser. The solvent was heated to reflux. The solvent vapour traveled up a distillation arm, and flooded into the chamber housing the thimble of sample. The condenser allows vapour to cool and drip back into solid material. The chamber was slowly filled with warm solvent. When the chamber becomes almost full, it was automatically emptied by a siphon side arm, with the solvent running back to the distillation flask. This cycle was allowed to repeat many times, till the colourless liquid was seen through a siphon side arm.
After extraction the distilled water was removed, typically by means of distillation apparatus yielding the extracted compound. It was later dried over water bath and hot air oven. 5120 mg of this *kasisadi varti* aqueous extract was dissolved in 10ml of dimethyl sulfoxide (DMSO) and the stock solution was further diluted to required concentration as 512mg/ml, 256mg/ml, 128mg/ml, 64mg/ml, and 32mg/ml.

Preparation of Culture media (19)

Preparation of Sabouraud Dextrose Broth

The following ingredients dextrose 40g, beef extract 5 g, casein peptone 5 g were taken and dissolved in 1000 ml distilled water and Ph was adjusted to 5.6±0.2. Then media was autoclaved at 121°C for 20 minutes.

Preparation of Sabouraud Dextrose Agar Medium (SDAM)

The following ingredients dextrose 40g, beef extract 5 g, casein peptone 5 g were taken and dissolved in 1000 ml distilled water and pH was adjusted to 5.6±0.2 and 15 g of agar was added, mixed. Then media was autoclaved at 121°C for 20 minutes.

Preparation of Test organism

Candida albicans was procured after developing culture from the vaginal smears. The smears were taken from female patients and diagnosis was confirmed by presence of pseudohyphae of Candida. The positive twenty results of *Candida albicans* were selected for the study. Their sensitivity was tested against the aqueous extract of the drug *kasisadi varti*.

Wet mounting of vaginal sample KOH mount (20)

Specimen was placed on a slide to which a drop of 10-20% KOH was added and then covered by a coverslip and left for 20 minutes in incubator at 37°C to digest keratin. KOH dissolves keratin and cellular material but does not affect fungi.

Culturing of vaginal swab: streak culture (surface plating) (19)

Vaginal swabs from the diagnosed patients were collected after taking due consent. The vaginal swab was cultured by streaking method for subculture of fungi. The surface of SDA medium in petridish was inoculated with the specimen. The primary inoculation was made by swab. Once the primary inoculums were made, a loop was used for spreading the material into four quadrants of the plate. The plate was inoculated with the test organism by streaking the swab in a back and forth motion very close together as the plate is moved across and down. The plate was rotated to 60° and the above action was repeated. The plate was rotated once more and streaking action was repeated. The plate is incubated at 37°C for overnight. Confluent growth occurred at the primary site of inoculation and well separated colonies appeared on the final series of streaks.

Gram's staining Method (21)

Primary staining of heat fixed smear of specimen (vaginal swab) was made with a pararosaniline dye

(crystal violet, gentian violet solution) for one minute. Crystal violet and dilute solution of iodine, gram's iodine is poured off and kept for one minute. Washed with water. Decolourisation with an organic solvent is done for 10-30 seconds. Later washed with water. Counter stain with a dye of contrasting colour (dilute carbolfuchsin, safranin or neutral red) for 20-30 seconds.

Preparation of Standard drug (19)

50mg of Amphotericin B was dissolved in 2ml of Dimethyl Sulfoxide (DMSO). This stock solution was diluted to the concentration of 25mg/ml.

Determination of antifungal activity

The antifungal activity of aqueous extract of *kasisadi varti* (KV1, KV2, KV3) was done using agar well diffusion method. The work place was cleaned in laminar air flow using 70% ethyl alcohol and the UV for 20 minutes. One loop of *Candida albicans* was inoculated from the 48 hrs culture into 10 ml of Sabouraud dextrose broth and mix well. Around 15 ml of the Sabouraud dextrose agar media was poured uniformly over the sterile petridish. 1 ml of Sabouraud dextrose broth containing the fungus was added uniformly over petridish and mixed well and the media was allowed to solidify for 30 minutes. Seven equidistant wells were made on the plate with the help of sterile cork borer(1cm in diameter) 100 microlitre of the standard (Amphotericin B) and extract was added to the respective wells. Test was conducted for different concentrations of extract (32-512mg/ml) for three samples separately. All the petridishes were incubated at 37°C for 48-72 hrs. After the incubation period, the zone of inhibition was measured with a ruler.

The test was repeated twenty times, using culture of *Candida albicans* from different vaginal smears.

Determination of antifungal activity on MTCC strain of *Candida albicans*

As an extended study, the antifungal activity was done on MTCC strain of *Candida albicans*

Preparations of yeast extract dextrose agar media (19)

Yeast extract (3 g), peptone (10 g) and dextrose (20 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.4 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121°C for 20 minutes.

Preparation of the inoculum (19)

Candida albicans (MTCC 183) was procured from culture collection centre, IMTECH, Chandigarh. Loopful of 48 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Well diffusion method (19)

The media was cooled to around 45-55°C, around 20 ml each was poured into sterile petriplates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 30°C and observed after 48 h.

Observations and Results

Result of in vitro study of aqueous extract of *Kasisadi varti* (KV1, KV2, KV3)

Table 2: Zone of inhibition obtained by In vitro antimicrobial sensitivity test for *Kasisadi varti* (KV1. KV2, KV3) against the *Candida albicans* strain cultured from vaginal smear

Patient Sample		ZONE OF INHIBITION in mm					
		512 mg/dl	256 mg/dl	128 mg/dl	64 mg/dl	32 mg/dl	Standard (Amphotericin B)
1	KV1	9	0	0	0	0	0
	KV2	9	0	0	0	0	0
	KV3	10	8	0	0	0	7
2	KV1	12	10	0	0	0	0
	KV2	8	6	0	0	0	0
	KV3	12	0	0	0	0	0
3	KV1	19	14	10	0	0	0
	KV2	10	7	0	0	0	6
	KV3	15	10	0	0	0	0
4	KV1	0	0	0	0	0	0
	KV2	8	0	0	0	0	0
	KV3	13	0	0	0	0	0
5	KV1	19	12	9	0	0	0
	KV2	9	0	0	0	0	0
	KV3	18	13	10	0	0	0
6	KV1	10	0	0	0	0	0
	KV2	12	0	0	0	0	0
	KV3	8	0	0	0	0	0

7	KV1	11	15	0	0	0	0
	KV2	9	0	0	0	0	0
	KV3	11	10	0	0	0	0
8	KV1	14	14	8	0	0	6
	KV2	11	0	0	0	0	6
	KV3	12	12	9	0	0	0
9	KV1	20	16	12	8	0	14
	KV2	11	8	0	0	0	8
	KV3	18	16	11	8	0	0
10	KV1	17	15	11	0	0	0
	KV2	9	8	0	0	0	0
	KV3	10	12	8	0	0	0
11	KV1	10	8	0	0	0	0
	KV2	11	12	0	0	0	0
	KV3	14	12	9	0	0	0
12	KV1	35	30	24	18	15	12
	KV2	25	18	12	8	7	9
	KV3	30	28	25	20	18	12
13	KV1	17	14	12	12	0	0
	KV2	12	10	0	0	0	0
	KV3	20	20	17	12	10	7
14	KV1	17	10	0	0	0	7
	KV2	18	14	6	0	0	6
	KV3	18	14	12	10	0	6
15	KV1	30	28	24	20	18	14
	KV2	28	25	20	17	15	0
	KV3	32	26	26	24	23	0
16	KV1	30	27	23	20	16	7
	KV2	24	22	18	12	12	12
	KV3	30	26	25	24	20	9
17	KV1	19	15	12	10	0	0
	KV2	20	14	0	0	0	0
	KV3	18	14	14	0	0	8
18	KV1	20	24	16	10	8	0
	KV2	24	15	11	0	0	0
	KV3	28	24	18	15	12	11
19	KV1	17	14	10	0	0	0
	KV2	13	0	0	0	0	0
	KV3	19	15	11	9	0	12
20	KV1	25	18	15	12	8	12
	KV2	15	10	0	0	0	11
	KV3	20	17	14	10	0	12

B. Result of experimental study on MTCC strain of *Candida albicans*

Table 3:Result of invitro antifungal activity of *Kasisadi varti* against MTCC strain of *Candida albicans*

Concentration	ZONE OF INHIBITION in mm		
	KV 1	KV2	KV3
512mg/dl	0	0	0
256mg/dl	0	0	0
128mg/dl	0	0	0
64mg/dl	0	0	0
32mg/dl	0	0	0
Control (D.W)	0	0	0
Standard (Amphotericin B)	10	10	10

Statistical Analysis

To interpret the above results, one way anova test with Post Hoc Multiple comparison test was applied. The Mean (M), Standard deviation (S.D) and Standard Error (S.E) was tabulated as follows-

Table 4: Result of Descriptive Analysis (Mean, Standrad deviation and Standard Error)

Concentration	KV1			KV2			KV3			Order of mean
MG/ML	M	S.D	S.E	M	S.D	S.E	M	S.D	S.E	
32	3.25	6.16	1.38	1.70	4.35	0.97	4.15	7.79	1.74	KV3>KV1>KV2
64	5.50	7.51	1.68	1.85	4.75	1.06	6.60	8.57	1.92	KV3>KV1>KV2
128	9.30	8.37	1.87	3.35	6.49	1.45	10.45	8.77	1.96	KV3>KV1>KV2
256	14.20	8.59	1.92	8.45	7.88	1.77	13.85	8.28	1.85	KV1>KV3>KV2
512	17.55	8.18	1.83	14.30	6.46	1.45	17.80	7.22	1.61	KV3>KV1>KV2
Standard	3.60	5.40	1.20	3.60	5.40	1.20	3.60	5.40	1.20	Not applicable

M-Mean; S.D- Standard Deviation; S.E-Standard Error

Table 5: Result of ONE WAY ANOVA

Conc. (mg/ml)	Comparision	Sum of Squares	Df	Mean Square	F	P	Significance
32	Between Groups	80.138	3	26.713	0.752	p>.05 (0.524)	Not Significant
	Within Groups	2699.050	76	35.514			
64	Between Groups	251.050	3	83.683	1.893	p>0.05 (0.138)	Not Significant
	Within Groups	3358.900	76	44.196			
128	Between Groups	769.937	3	256.646	4.811	p<0.05 (0.004)	Significant
	Within Groups	4054.250	76	53.345			
256	Between Groups	1379.637	3	459.879	8.036	p<0.05 0.000	Significant
	Within Groups	4349.250	76	57.227			
512	Between Groups	2458.900	3	819.633	17.702	p<0.05 0.000	Significant
	Within Groups	3518.900	76	46.301			

df-degree of freedom,p-probability value, groups implies the three samples of *kasisadi varti* and the standard drug.

Discussion

In the present investigation, the antifungal activity of the aqueous extract of *kasisadi varti* was assayed against microorganism *Candida albicans* cultured from patient's sample. The assay was done at different concentrations of the extract using Amphotericin B as standard to understand the most effective activity.

The *Candida albicans* was cultured from the different vaginal swabs from female patients. The positive twenty results of *Candida albicans* was selected for the study. Their sensitivity was tested against the aqueous extract of the drug *kasisadi varti*. The results of the antimicrobial assay indicated that drug exhibited sensitivity against the tested microorganisms at different concentration that is 32mg/ml, 64mg/ml, 128mg/ml, 256mg/ml, 512mg/ml with a zone of inhibition of 0 to 35mm. The maximum zone of inhibition was obtained at a concentration of 512mg/ml (35mm) by KV1, By KV2 at 512mg/ml (28mm) and by KV3 at 512mg/ml (32 mm).The zone of inhibition shown by standard drug varied from 0 mm to 14 mm at concentration of 25mg/ml.

In multiple comparison test (Post Hoc test), at concentration 512mg/ml, all three samples KV1, KV2 and KV3 showed statistically significant difference against standard drug(Amphotericin B). But statistically significant difference was not seen between KV1, KV2 and KV3. This indicates that all three samples showed better zone of inhibition than the standard drug used. But among the three groups difference could not be highlighted statistically.

At concentration 256mg/ml, KV1 and KV3 showed statistically significant difference against standard drug. KV2 did not show statistically significant difference against standard. And no statistical significant difference among KV1, KV2 and KV3 was seen. This indicates that KV1 and KV3 showed better zone of inhibition compared to standard drug.

At concentration 128mg/ml, only KV3 showed statistically significant difference against standard drug. Also KV3 showed statistically significant difference when compared to KV2. No statistical significant difference between KV1 and KV2, or KV1 and KV3. At this concentration only KV3 showed better zone of inhibition when compared to standard drug and KV2. But difference could not be highlighted either among KV1 and KV2, or KV1 and KV3.

At concentration 64mg/ml and 32mg/ml, statistically significant difference was not seen among any group and against standard.

In comparative view of different samples of *kasisadi varti*, better results was seen in KV3, as statistically the mean value of zone of inhibition against *Candida albicans* shown by KV3 was more than KV1 and KV2 at the concentration of 32mg/ml, 64 mg/ml, 128mg/ml and 512 mg/ml. i.e., 4.15 mm, 6.60mm, 10.45mm and 17.80mm respectively.

Significant results in all three groups were obtained at the concentration of 128mg/ml, 256mg/ml and 512mg/ml in multiple comparison test (Post Hoc test) proving antifungal activity when compared to standard drug.

The difference in zone of inhibition among the twenty samples in same *varti* group (KV1, KV2, KV3) may be because the Candida samples obtained from different patients might be different in species and virulence.

To understand the difference in the virulence of microorganism the study was further extended to next trial. The invitro study was performed on MTCC (Microbial Type Culture Collection) strain of *Candida albicans*. Here, *kasisadi varti* did not show any zone of inhibition against the fungi. The possible cause of negative result would be that the microorganism might be resistive or virulent than the microorganism in patient sample.

In vagina the strain of *Candida albicans* are of different types (22,23) and may differ from the MTCC strain. Also, the fungi may be less pathogenic in vagina compared to strain obtained (24). Hence good sensitivity was observed by the test drug against the microorganism of patient sample. The presence of alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids and steroids in terminalia chebula (25) is proved to be having antifungal activity.Terminalia bellerica(26), Emblica officinalis (27), Syzygium cumin (28), Magnifera indica (29), Woodfordia fruticosa (30) all have shown antifungal activity against *Candida albicnas.*

Conclusion

Kasisadi churna is a formulation mentioned for external application in *kaphaja yoni vyapath*. The *churna* was modified into *varti* form and prepared by three different methods. These three samples were tested experimentally for its action against *Candida albicans*. All three samples *bhavana* (KV1) method, *gudapaka* (KV2) and modified method (KV3) showed significant results on *Candida albicans* developed from vaginal smears of patients at a concentration of 128mg/ml, 256mg/ml, and 512 mg/ml in comparison with standard drug amphotericin B. Among the three groups cocoa butter method (KV3) has shown better effect than *bhavana* (KV1) and *gudapaka* (KV2) method. None of the samples showed any effect on mtcc strain of *Candida albicans*.

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