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Comparative evaluation of antifungal activity of Vidanga Taila, Katu Taila & Murchhita Katu Taila against candida albicans

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Abstract
As per literature of Ayurveda there are tradition of medical practices of Sneha Kalpana to cure and mitigate diseases and disorders. Aacharyas treats various kind of diseases comprises antifungal effects. One of the important Sneha Kalpana formulation named Vidanga taila was traditionally employed for the cure & mitigation of the Krimiroga such as Yuka, Liksha and other types of Bahyakrimiroga. Among all these described diseases Yuka, Liksha might be due to infection of fungal growth so, it is compulsory to investigate the antifungal activity of Vidanga taila, Katu taila (Mustard oil) and Murchhita Katu taila (Murchhita mustard oil). This current study was carried out with the aim of assessing comparative evaluation of Antifungal activity of Vidanga taila, Katu taila & Murchhita Katu taila against Candida Albicans. This study showed that Vidanga taila sample A, Vidanga taila sample B and Vidanga taila sample C was found most effective oil against Candida albicans in comparison to Katu taila (Mustard oil) and Murchhita Katu taila (Murchhita mustard oil) it is due to the ingredients {Vidanga (Embelia ribes Burn), Gandhaka (sulphur), Manahshila (Realgar), Katu taila (Mustard oil) and Gomutra (cow'urine)} of Vidanga taila which have the antifungal activity.

Keywords: Vidanga taila, Katu taila, Murchhita Katu taila, antifungal sensitivity test (AST), minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and zone of inhibition (ZOI).

Introduction
Since ancient time presence of different types of Krimis [1] were very well understood by Aacharyas. On the basis of causes, sign, symptoms and line of treatment Krimiroga which is described by Ayurvedic Aacharyas is comparable with fungal diseases. The purpose of this current study is to investigate Vidanga tail [2] action on ringworm infestation. Regarding this fungus Candida albicans have been followed. In current situation of exposure of drug resistance and ill side effects caused by modern medicines shows the urgency of Ayurvedic medicines to overcome the complication of the Krimiroga. Potent Vidanga taila is very effective to cure the diseases and disorder related to Krimiroga as compared to other Kriminasaka formulations.

Aim
The basic ground of this current study is to determine the in-vitro antifungal activity of Vidanga taila, Katu taila and Murchhita Katu taila also to evaluate its potency in cure & mitigation of ringworm infestation caused by candida albicans as a traditional Ayurvedic medicament.

Materials and Methods
To evaluate the antifungal sensitivity test of the Vidanga taila sample A, a micro dilution technique was used. The fungal spores was washed from the surface of agar plates with sterile 0.85% saline consisting 0.1% Tween 80 (v/v). The spore suspension was adapted with sterile saline to a concentration of about 1.0 × 107 cfu/ml. The inocula was kept at 4 ° C for supplementary application. Dilutions of the inocula was cultured on solid potato dextrose agar to authenticate the non-appearance of contamination and to examine the authentication of the inoculum.

The same procedure was repeated for the Vidanga taila sample B, Vidanga taila sample C, Katu taila (Mustard oil) and Murchhita Katu taila (Murchhita Katu taila). Which has been tabulated in table No 1. [3] Inoculum preparation for minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFC) [3] Inocula were achieved through an overnight agar culture of the tested organism (Candida albicans). Inoculum for the MIC and MFC test was obtain by way of taking atleast five well isolated colonies of the matched morphology through agar plate culture. The top of each colony was touched by a sterile loop & the loop was transfer into a tube that has 5 ml of normal saline and then vortexes. The broth culture was incubated at temperature 37 °C and noticed for about 4 hrs. Till it achieve the turbidity of 0.5 McFarland standard (1.5 × 108 cfu/ml).

Determination of MIC: [4] Minimum inhibitory concentration (MIC) of the Vidanga taila sample A was done through the agar well diffusion method. The Nutrient broth of quantity 2ml possess the test organism (Candida albicans) was dispensed into a sterilized test tube. A quantity of 1ml of Vidanga taila sample A at a concentration of 100 µl was added to the test tubes containing the test organism (Candida albicans). The tube was corked and incubated aerobically at temperature 37 °C for 24 hrs. The tube was investigated after incubation and MIC was evaluated. The same procedure was repeated for Vidanga tail sample B and Vidanga tail sample C, Which has been tabulated in table number 2 and shown in graph no. 1.

Determination of MFC: [5] The minimum fungicidal concentration (MFC) was investigated by sub culturing of 2 µl from each of the wells that shown no growth into microliter plates consisting 100 µl of broth contain in each well and further incubated for 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. Fluconazole (commercial standards) was used as positive control (10 µg/ml) [6] and negative control {Dimethyl Sulfoxide (DMSO—99.9%)} for fungus (Candida albicans). These experiments were accomplished in duplicate and repeated three times for reproducibility. The same procedure was repeated for Vidanga tail sample B and Vidanga tail sample C, Which has been tabulated in table no. 2 and shown in graph no 1. To determine the ZOI commercial standards, fluconazole, was used as positive control (10 µg/ml) and negative control {DMSO-Dimethylsulfoxide (DMSO)-99.9%} for fungus (Candida albicans).

Results

Comparative evaluation of Antifungal activity of Vidanga taila, Katu taila & Murchhita Katu taila against Candida albicans were performed by the experiment mentioned in NCCLS (National Committee for Clinical Laboratory Standards) guideline/ CLSC (Clinical and Laboratory Standards Institute [7]. That includes antifungal sensitivity test (AST) [8], minimum inhibitory concentration (MIC) [9], minimum fungicidal concentration (MFC) [10] and zone of inhibition (ZOI) [11]. The AST values shows the capacity of a specific organism (Candida albicans) to expand in the presence of a particular drug *in- vitro*. The tested samples of Vidanga taila sample (A, B and C) Katu taila (Mustard oil) and Murchhita Katu taila (Murchhita mustard oil) sequence indicates AST values that are tabulated in table no. 1 are as 1.2, 1.5, 2.4, 0.4 and 0.9. Evaluating these obtained AST values it can be concluded that Vidanga taila sample C was more sensitive for tested organism (Candida albicans) as compared to other tested samples.

MIC value shows the minimum quantity of samples that statics the growth of fungus (Candida albicans). Among the tested samples of Vidanga taila sample A, Vidanga taila sample B and Vidanga taila sample C indicates MIC values which are indicated in graph no.1 & tabulated in table no. 2 are as 400 µl, 200 µl, 50 µl. Evaluating these obtained MIC values it can be concluded that Vidanga taila sample C has more statics activity on tested organism (Candida albicans) as compared to other tested sample.

MFC values shows that concentration which kill the fungus. Among the tested samples of Vidanga taila sample A, Vidanga taila sample B and Vidanga taila sample C indicates MFC values which are shown in graph no.1 & tabulated in table no. 2 are as 600 µl, 500 µl, 200 µl. Evaluating these obtained MFC values it can be concluded that Vidanga taila sample C has more effective concentration that killed the tested organism (Candida albicans) as compared to other tested sample.

The ZOI is defined as the area on an agar plate where the growth of fungus is block in the presence of an antibiotics or the other antimicrobial compound. The tested samples of Vidanga taila sample (A, B and C,) Katu taila (Mustard oil), Murchhita Katu taila (Murchhita mustard oil) & fluconazole sequence shown ZOI values (mm). Which are shown in figure. no. 1, graph no. 2 & tabulated in table no. 3 are as 8.83±0.17, 10.33±0.33, 13.33±0.88, 5.83±0.60, 3±0.58 and ± 1.20 at concentration of 100 µl. Evaluating these obtained ZOI(mm) values it can be concluded that Vidanga taila sample C was more effective for tested organism (Candida albicans) as compared to other tested samples.

Statistical Analysis

Means and standard error of the mean were calculated for the zones of inhibition (mm) which have been measured and values are expressed as mean ±SEM and analysed by one way analysis variance (ANOVA) followed by Dennet's multiple comparison; P**0.0076, P**** < 0.0001, fluconazole (commercial standard) was used as positive control (10 µg/ml) and negative control {DimethylSulfoxide (DMSO - 99.9%)} for fungus (Candida albicans). The comparative values of zone of inhibition (mm) for samples of Vidanga taila A, B, C, Katu taila (Mustard oil) and Murchhita Katu taila (Murchhita mustard oil) have been tabulated in table no. 3, shown in graph no. 2 and also figured in fig no 1.

Table 1: AST observation of Vidanga Taila, Katu Taila and Murchhita Katu Taila

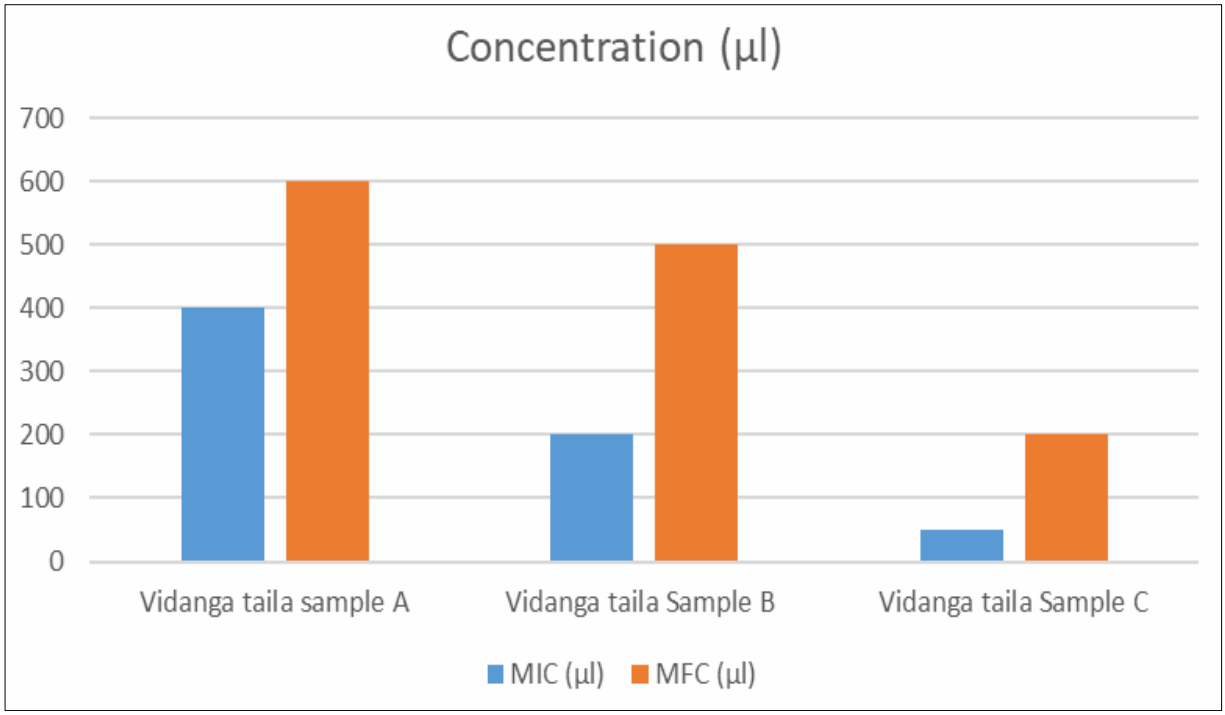
Sr. No.	Tested Samples	Tested fungus	Antifungal Sensitivity Test (AST)
1.	Vidanga taila sample A	Candida albicans	1.2
2.	Vidanga taila sample B	—	1.5
3.	Vidanga taila sample C	—	2.4
4.	Katu taila(Mustard oil)	—	0.4
5.	Murchhita Katu taila (Murchhita mustard oil)	—	0.9

Table 2: Mic and MFC observation of Vidanga Tailasample

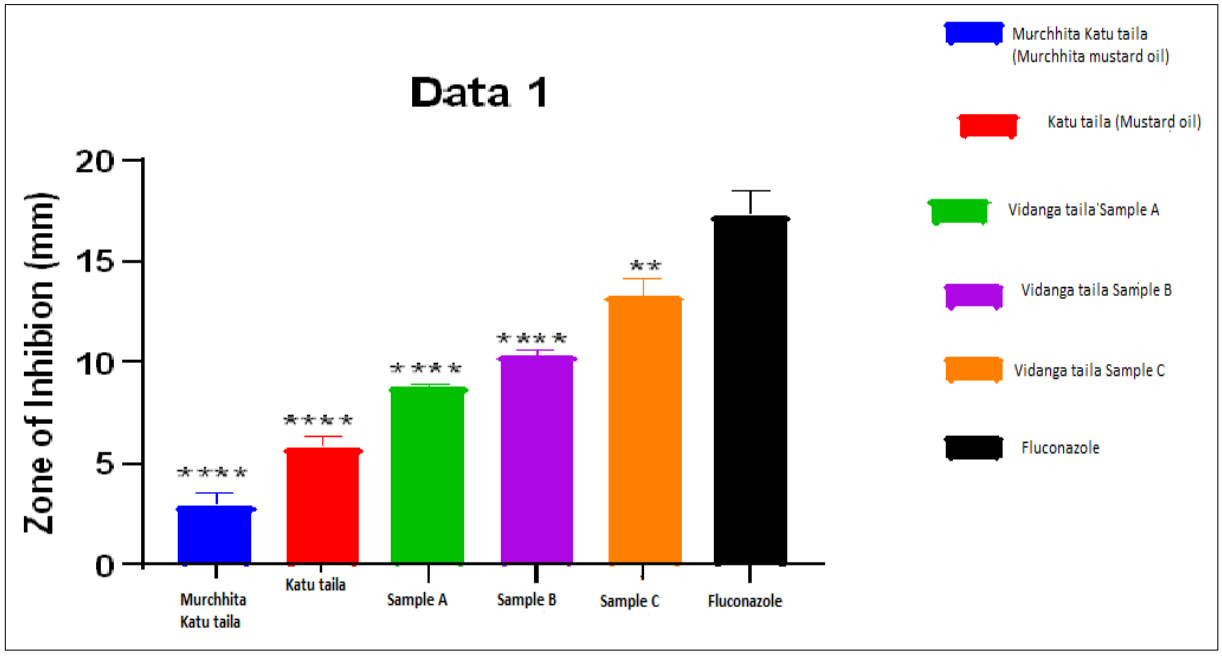
Sr. No.	Tested Samples	Minimum Inhibitory Concentration (MIC)	Minimum Fungicidal Concentration (MFC)
1.	Vidanga taila sample A	400 µl	600 µl
2.	Vidanga taila sample B	200 µl	500 µl
3.	Vidanga taila sample C	50µl	200µl

Table 3: Zone of Inhibition of Vidanga Taila, Katu Tailmurchhita Katu Taila and Fluconazole

Sr. No.	Tested Fungus	Tested Sample	Concentration	Zone of Inhibition (mm.)
1.	Candida albicans	Vidanga tail sample A	100 µl	8.83±0.17
2.	—	Vidanga tail sample B	100 µl	10.33±0.33
3.	—	Vidanga tail sample C	100 µl	13.33±0.88
4.	—	Katu tail (Mustard oil)	100 µl	5.83±0.60
5.	—	Murchhita Katu tail (Murchhita mustard oil)	100 µl	3±0.58
6.	—	Fluconazole	100 µl	17.33±1.20



Graph 1: Showing MIC and MFC of Vidanga Tailasample



Graph 2: Showing zone of inhibition of Vidanga Taila, Katu Tail, Murchhitakatu Taila and Fluconazole

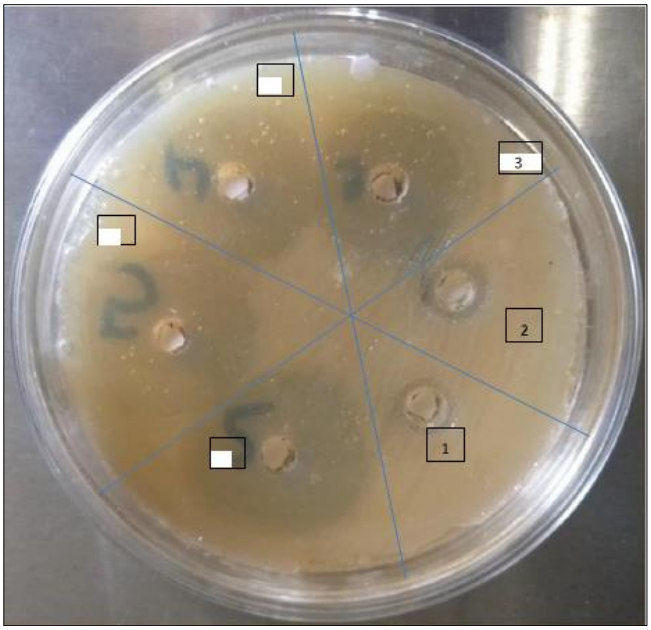


Fig 1: Zone of inhibition (mm.) of tested samples: 1. Murchhita Katu Taila, 2. Katu Taila, 3. Vidanga Taila Sample C, 4. Vidanga Taila Sample B 5. Vidanga Taila Sample A & S. Standard Fluconazole

Discussion

As per comparative evaluation of Antifungal activity of Vidanga taila, Katu taila & Murchhita Katu taila against Candida albicans by the means of antifungal sensitivity test, minimum inhibitory concentration, minimum fungicidal concentration and zone of inhibition it can be strongly committed that Vidanga taila sample A, Vidanga taila sample B and Vidanga taila sample C was found most efficacious towards Candida albicans in comparison to Katu taila (Mustard oil) and Murchhita Katu taila (Murchhita mustard oil) this is by virtue of the {Vidanga(Embelia ribes Burn), Gandhaka (sulphur), Manahshila (Realgar), Katu taila (Mustard oil) and Gomutra (cow'urine)} of Vidanga taila that possesses antifungal activity. The difference between the antifungal effect of Katu taila (Mustard oil), Murchhita Katu taila (Murchhita mustard oil) and Vidanga taila was noticed because of the cumulative potency of all the ingredients with Katu taila (Mustard oil). The pharmacodynamics of Vidanga taila can be elucidated for the cure and mitigation of fungal disease on skin as the keratinized property of the skin is primarily map out to block desiccation so, the skin is a relatively lipophilic/ hydrophobic barrier. Afterward both oils and the skin (epidermis) are relatively lipophilic, they mix relatively well together; Therefore, oils retain prominent propensity for the transdermal absorption [12-15]. Because of the transdermal absorption the Vidanga taila invade the inside of the skin and delivered the therapeutics action on the fungus and cure the skin diseases & disorders such as Yuka, Liksha, and other types of the Bahya Krimis.

Conclusion

A Herbo-mineral formulation named as Vidanga taila retain very effective therapeutic applications. Which has been recertified by means of its antifungal study. So, it can be applied for cure and mitigation of numerous varieties of Bahya Krimi such as Yuka, Liksha. By doing this current study it can be said that Vidanga taila is very effective Ayurvedic medicament for Bahya Krimi such as Yuka, Liksha {antifungal action as on ringworm (Candida albicans)}. New future coming more and more research works needs to be performed on Vidanga taila by the means of different animal model along with different types of standard approved parameters to determine its more and more therapeutic potential in the field of clinical practices. Still numerous analysis and investigation are required for proper identification of all the active chemical constituents of the Vidanga taila. To further investigate its therapeutic profile with reference to fungal diseases clinical trials may be conducted.

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