Original Research Paper

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Oral Pathology

EFFICACY OF ANTI-FUNGAL PROPERTY OF THE EXTRACT OF DAHLIA PINNATA ON CANDIDA ALBICANS- AN IN VITRO STUDY.

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ABSTRACT **Context:** Oral thrush or Candidiasis remains a common problem in children, elderly and immunocompromised patients with ailments like renal diseases, diabetes mellitus etc. Candida albicans is the main causative organism for this disease. Long term use of chemical antifungal make Candida albicans resistant to them. Hence more effective methods/drugs are required to combat Candida.In present scenario research works are being carried out to seek more traditional or herbal treatment to have better healing effects without adverse effects of the synthetic antifungal drugs that are been currently used. **Aims:** To determine minimum zone of inhibition of Dahlia pinnata against C.albicans **Settings and Design:** An Invitro Study. **Methods and Material:** The well diffusion method using Sabouraud dextrose agar plates was used to evaluate the anticandidal activity of 5%, 10% and 50% concentration of Dahlia pinnata leaves extract against C.albicans (ATCC 90228) in comparison with 5% Fluconazole. **Statistical Analysis :** Results were analyzed using SPSS version 19. Independent sample t test was used to compare mean zone of inhibition between two groups with the P value <0.05 was considered statistically significant. **Results:** Dahlia pinnata leaves extract exhibited the inhibitory effect against C.albicans. This inhibitory effect was found to increase as the concentration of the plant extract increased with 5% showing 2.103mm, 10% showing 3.057mm and 50% showing 5.127mm; however even at the highest concentration, it was much less compared to 5% concentration of Fluconazole (6.99mm). **Conclusions:** The anticandidal activity of Dahlia pinnata leaves against C.albicans needs to be further studied, which may increase its possibility of getting used in herbal medicine in the near future.

KEYWORDS : Dahlia pinnata ,Candida albicans, Ethanolic extract, Efficacy

INTRODUCTION

Oral thrush is a common pathological condition that is not identified easily first. It is usually harmless and painless, but can lead to serious problems. “Oral candidiasis” or Oral thrush is most often caused by a type of fungus called Candida albicans found generally in the mucous membrane of our oral cavity as normal commensal, which may become invasive in immunocompromised individuals. It usually affects children and immunocompromised people as in diabetes mellitus, renal diseases and other comorbidities.[1]

Oral candidiasis is treated most effectively by topical drugs like nystatin and amphotericin whereas fluconazole is the drug of choice for systemic treatment. Studies have shown that fluconazole oral suspension also to be a very effective drug in the treatment of oral candidiasis due to its good antifungal properties, its high acceptance by the patient and its efficacy compared with other antifungal drugs.[2]

The synthetic drugs might have side effects like toxicity, possibility of drug interactions or pathogens developing resistance leading to its ineffectiveness. So there is always a need to search for a safer and economically viable alternative from various sources including plants and herbs. Strong antifungal activity against C.albicans have been exhibited by many plant extract like Lawsonia inermis, Pelargonium graveolens, Camellia sinensis, Mentha piperita, Citrus latifolia and plants belonging to asteraceae family.[3]

Dahlia belongs to the family asteraceae and is a perennial herbaceous plant producing stems up to 1 metres tall from a tuberous rootstock. More than twenty species of Dahlia have been described which includes Dahlia variabilis and Dahlia pinnata. Primarily, Dahlia has been utilized as an ornamental plant for its attractive flowers. However the plant parts like flowers, leaves, seeds and roots are edible and are used locally as a food or as dyes.[4]

Medicinal uses of various parts of Dahlia have been reported. The root is rich in starch insulin, unabsorbed by body but converted into fructose, which is suitable for diabetics as sweetening substance.[5] Dahlia pinnata has five foliate leaves and large single flower of bluish red color.The antibacterial activity of both fresh and dried plant parts of Dahlia pinnata have been tested effectively against E.coli, Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogenes and Agrobacterium tumefaciens using agar disc diffusion method with it's various extracts.[6]

Dahlia variabilis tuber extract has has been effectively used against pathogenic skin fungi C.albicans and M.gypsum.[5] Dahlia flower extract has shown antimicrobial activity against cariogenic pathogens like S.Mutans, S.Aureus, L.Acidophilus and S.gordinii.[7]

However there is no study on Dahlia pinnata leaves extracts against C.albicans, so the aim of this study was to find the antifungal property of Dahlia pinnata leaves extract against C.albicans and hence to prevent oral candidiasis in a traditional way.

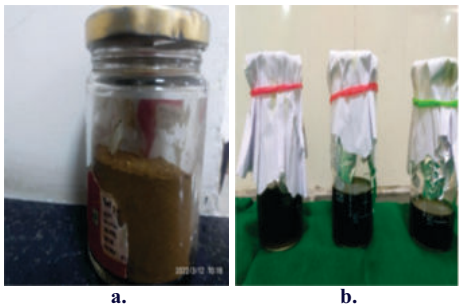
Subjects and Methods

1. Ethanolic extract of 5%, 10%, and 50% concentration of Dahlia pinnata leaves procured from local nursery.
2. Ethanol
3. Fluconazole-150 mg tablets
4. Candida Albicans strain (ATCC 90228) .
5. Sabaroud Dextrose Agar
6. Sabouraud Dextrose Broth
7. Scale and Vernier calipers for measuring zone of inhibition
8. Digital scale and Digital Centrifuge-5000 RPM

Methodology

This study was conducted after the approval from the Institutional Ethics Committee. The Dahlia pinnata plant was procured from a local nursery in Bangalore. The verification of plant and species was done by the horticulturist at Association for People with physical Disability, JB Nagar, Bangalore.

Preparation of Dahlia pinnata Plant leaves Extract:[7,8]

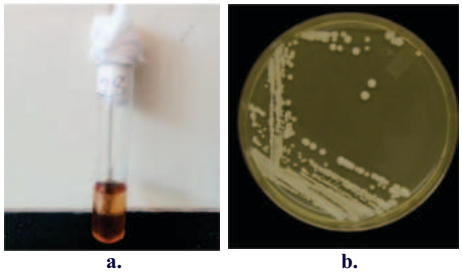


a. b.

Figure 1: (a) The dried leaves were grounded well into a fine powder (b) The ethanol extract was prepared at 5%, 10%, 50% concentration and was stored at 20 degree Celsius.

The selected Dahlia pinnata plant leaves were thoroughly washed with running water followed by distilled water and then dried under shade at 28± 2°C for about 10 days. The dried leaves were grounded well into a fine powder (figure 1a) in a mixer grinder and sieved. The powder was stored in air sealed polythene bags at room temperature before extraction. This was weighed into 5gm, 10gm, 50gm,) and was transferred to sterile beakers. To each beaker 100 ml of ethanol was added and soaked for 48 hours. This solution was centrifuged at 2000 rpm for 10 minutes and then the supernatant was filtered using a 0.45mm filter membrane. The ethanol extract was prepared at 5%, 10%, 50% concentration and was stored at 20 degree celsius (figure 1b).

Preparation of Culture Media



a. **b.**

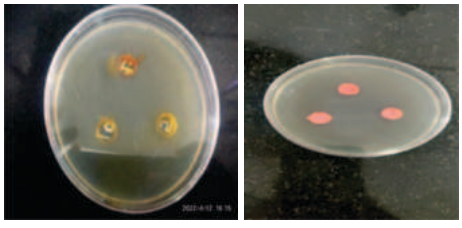
Figure 2: (a) The strains of C.albicans cultured in Sabouraud Dextrose broth by incubation at 37 degree Celsius for 48 hours and (b) then was streaked onto the Sabouraud Dextrose agar plate using loop for a nutritious growth.

The strains of C.albicans cultured in Sabouraud Dextrose broth (figure 2a) by incubation at 37 degree Celsius for 48 hours and then was streaked onto the Sabouraud Dextrose agar plate using loop for a nutritious growth (figure 2b).

Preparation of Fluconazole

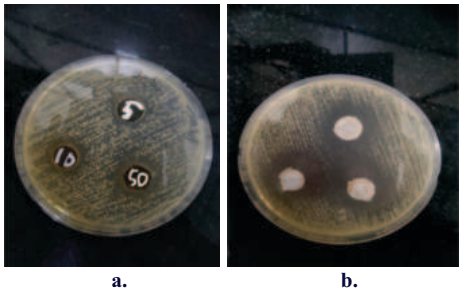
10 Fluconazole tablets, each of 150 mg were taken and grinded to make a fine powder of 1500 mg. 30 ml of water was added to the powder and mixed thoroughly to make a 5% aqueous solution of fluconazole.

Ditch Plate Method



a. **b.**

Figure 3 (a) Wells were filled with extracts of various concentrations, separate plates for Dahlia pinnata leaves extract with different concentrations (b)and fluconazole were filled.



a. **b.**

Figure 4 (a) the zone of inhibition of Dahlia pinnata leaves extract and (b) fluconazole were measured in millimetres using Vernier callipers.

The solid agar plates (60 numbers) were streaked under laminar flow cabinet and were punched with wells having a diameter of 7 mm and the wells were filled with extracts of various concentrations. Separate plates for Dahlia pinnata leaves extract with different concentrations (figure 3a) and fluconazole (figure 3b) were used. The plates were incubated at 37°C for 48h. After incubation, the zone of inhibition of Dahlia pinnata leaves extract (figure 4a) and fluconazole (figure 4b) were measured in millimetres using Vernier callipers.

RESULTS

Data was entered in Microsoft Excel sheet and was analysed using the statistical package for the Social Sciences (version 19). Continuous variables are presented as Mean±SD . Levene's test was performed to test homogeinity of variances of data. Since the minimum zone of inhibition was normally distributed at 5%, 10% and 50% between the extract and 5% Fluconazole group, an Independent sample t test was performed to compare the mean minimum zone of inhibition at 5%, 10%, 50% between the group. P value<0.05 was considered as statistically significant.

Analysis Of Results:

Independent Samples T-test

Table 1 Group Statistics

Groups		N	Mean	Std. deviation	Std. mean error
5%	Dahlia pinnata	30	2.103	0.2220	0.0405
	Fluconazole	30	6.993	0.1721	0.0314
10%	Dahlia pinnata	30	3.057	0.1736	0.0317
	Fluconazole	30	6.993	0.1721	0.0314
50%	Dahlia pinnata	30	5.127	0.1081	0.0197
	Fluconazole	30	6.993	0.1721	0.0314

Table 2 Independent Samples Test

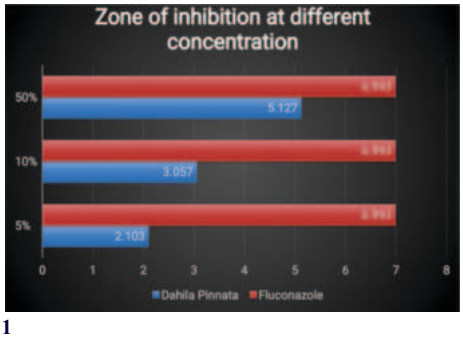
		Levene's Test for Equality of		t-test for Equality of	
5%		F	Sig.	t	df
	Equal variances assumed	2.037	0.159	-95.347	58
	Equal variances not assumed			-95.347	54.600
10%	Equal variances assumed	0.041	0.841	-88.221	58
	Equal variances not assumed			-88.221	57.996
50%	Equal variances assumed	5.245	0.026	-50.317	58
	Equal variances not assumed			-50.317	48.796

Table 3 Independent Samples Test

		t-test for Equality of Means		
		P value	Mean difference	Std. Error difference
5%	Equal variances assumed	0.000	-4.8900	0.0513
	Equal variances not assumed	0.000	-4.8900	0.0513
10%	Equal variances assumed	0.000	-3.9367	0.0446
	Equal variances not assumed	0.000	-3.9367	0.0446
50%	Equal variances assumed	0.000	-1.8667	0.0371
	Equal variances not assumed	0.000	-1.8667	0.0371

Table 4 Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
5%	Equal variances assumed	-4.9927	-4.7873
	Equal variances not assumed	-4.9928	-4.7872
10%	Equal variances assumed	-4.0260	-3.8473
	Equal variances not assumed	-4.0260	-3.8473
50%	Equal variances assumed	-1.9409	-1.7924
	Equal variances not assumed	-1.9412	-1.7921



Graph 1

Independent sample t test was performed to compare the means for two groups (Table 1)

There was a significant mean difference in zone inhibition concentration 5% between Dahlia pinnata extract and 5% Fluconazole as P value is <0.05 with mean difference -4.89.(Table 2)

There was a significant mean difference in zone inhibition concentration 10% between Dahlia pinnata leaves extract and 5% Fluconazole as P value is <0.05 with mean difference -3.93.(Table 2)

There was a significant mean difference in zone inhibition concentration 50 % between Dahlia pinnata leaves extract and 5% Fluconazole with mean difference -1.8Table 2)

The graph showing the comparison of mean zone of inhibition of Dahlia pinnata leaves extract and Fluconazole.(Graph 1)

DISCUSSION

Oral Candidiasis or thrush is an opportunistic fungal overgrowth and invasion of superficial tissues of oral mucosa. C.albicans an ubiquitous commensal organism of oral cavity is the main causative agent responsible for 95% of cases of oral candidiasis. The various attributes of C.albicans like, ablity to adhere to tissue surface and invade through secretion of enzymes and toxins, capacity to form biofilm on oral prosthesis and mucosa and mechanism to evade host defenses, help C.albicans to proliferate in oral cavity under altered host microenvironment. Three main classes of antifungals used for treatment of candidiasis are polyenes, azoles and echinicadins.[1]

The drug of choice in treatment of oral thrush is nystatin and amphotericin B as they have low hepatotoxcity however they have poor patient compliance due to unpleasant taste. Fluconazole as oral topical agent has been shown to be as effective as nystatin and amphotericin B and is better accepted by the patient.[2] In this present study 5% fluconazole was used as control.

One of the most significant shortcoming of synthetic drugs used as antifungals is their increased likelihood of developing resistance, and paucity of available classes of antifungal agents as treatment modalities.[1]

So there is an urgent need to explore alternative sources such as herbs and plants as medicines. Medicinal plants have been used as part of indian traditional systems of medicine since ancient times, and have been validated by modern scientific research on their phytochemicals. Studies on plants parts like barks, flowers and roots have demonstrated effectiveness in treating C.albicans. Furthermore studies have proven the efficacy of various solvents such as ethanol, methanol, chloroform, acetone and hexane for various plant parts extraction, to test their antimicrobial activity including against C.albicans.[7,8,9,10]

Though strains of C.albicans like ATCC 90028 and ATCC 10231 differ in type and amount of enzymes they produce, In vitro studies comparing the effect of various plant extracts on both strains have demonstrated similar inhibitory effects on pathogens. So in this present study C.albicans ATCC 90028 strain was used.[10,11]

Previous studies have shown plants belonging to asteraceae family exihibit good antimicrobial and anti candidal activity, hence in this present study different concentration of Dahlia pinnata leaves extract has been used as antifungal agent against C.albicans strain (ATCC 90028). While all the three concentrations showed inhibitory activity, the biggest zone of inhibiton of Dahlia pinnata leaves extract was measured with 50% concentration. These findings are similar to a study where instead of leaves, Dahlia flower methanol extract were used and it showed inhibitory activity against cariogenic pathogens including C.albicans.[7]

In the present study, the zone of inhibition increased as the concentration of Dahlia pinnata leaves extract increased, however this inhibition zone at the highest concentration of 50% was still less compared to 5% Fluconazole. These findings are comparable to those mentioned in a previous study, which demonstrated that though Dahlia flower extract has inhibitory effect against C.albicans, it was less compared to control of kanamycin.[7]

Limitations of the Study:

1. There is confusion in literature regarding Dahlia as a species. Existing studies on Dahlia mentioning Dahlia variabilis as a species, however we found that it is a generic name for all Dahlias.[4]
2. The Dahlia pinnata plants used in this study were procured from different nurseries, so there exists a possibility of variation in strains, though the plant species was authenticated by hortriculturist.
3. The original study was planned with nystatin tablets as control, however post pandemic due to its shortage, it was not available. So nystatin was substituted with Fluconazole. There are conflicting reports in literature regarding solubility of Fluconazole in water.[12-13] In our study we found that it dissolved easily.

CONCLUSION

As the C.albicans develop resistance to synthetic antifungal drugs, discovery of new plant extracts can serve as a possible source of substitute. The present study showed the effectiveness of antifungal activity of Dahlia pinnata leaves extract with ethanol solvent against C.albicans. One of the drawbacks of Dahlia pinnata plant is that being an ornamental plant, it is much in demand and is exorbitantly priced, nevethless it can be further researched for its use as effective antifungal.

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Key Messages: As Dahlia pinnata shows potent anticandidal activity against Candida albicans further studies can be performed to know more about its anticandidal activity.

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