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Formulation and Evaluation of Anti-fungal Herbal Lotion by using extract of *Catharanthus, Aloe-vera, Azadirachta indica* and *Vitex negundo*

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ABBREVIATIONS

C. albicans (Candida albicans), C. glabrata (Candida glabrata), C. parapsilosis (Candida parapsilosis), w/o (water-in-oil), o/w (oil-in-water), C. roseus (Catharanthus roseus), A. barbadensis miller (Aloe barbadensis miller), A. indica (Azadirachta indica), q.s. (quantity sufficient), °C (degree Celsius), Formulation 1 (F1), Formulation 2 (F2), Formulation 3 (F3), Micro-gram (µg), Millimeter (mm), Micro-litre (µL).

ABSTRACT

In today's daily life people show high interest in herbal medicine and herbal cosmetics due to their wide biological activities, high therapeutic efficacy, ease of availability, and minimum side effects as compared to the formulation of synthetic products. The present research work deals with the formulation and evaluation of herbal lotion for the treatment of antifungal disease caused by C. Albicans which is the most source of fungal infection in people as it caused approximately 90% of skin infections. In this study, we used an extract of C. roseus, Aloe vera, A. indica, and Vitex negundo with ethanol, glycerine, and coconut oil in different concentrations of F1 and F2. Thus, the herbal antifungal lotion formulation formulated was evaluated for its physicochemical parameters, in-vitro antifungal activity, and stability study of the formulation. All the herbal anti-fungal lotion formulations (as F1 & F2) showed positive results for physicochemical parameters with pH

Keywords: *Anti-fungal Herbal Lotion,* formulation of drugs, *physicochemical parameters.*

INTRODUCTION

In ancient times, Asian people traditionally used herbal plants to treat their disease and cure the disease. In India, Ayurveda is the most traditional system of medicine used by people for decades. In the last few decades, the use of herbal plants in the formulation of drugs and cosmetics has increased very instantly. Herbal plants are used in both fields, in the drug field to cure disease and as well as beauty products in the cosmetics field. Fungal infections, also called mycosis, are the most common disease that happens in people because of a weak immune system. The herbal plants attract researchers because of their tremendous therapeutic effects, having very less adverse effects, and being economical from the formulation's perspective. A lotion is a low-viscous pharmaceutical topical preparation intended for application on the skin. The pharmaceutical formulation of Creams and Gels is a most convenient form of topical formulation but it is not appropriate for application to hairy skin regions such as the scalp, underarm, and pelvic area where anti-fungal disease occurs more frequently. In this study, we are going to use extracts of some herbal plants like *C. roseus, A. barbadensis (Aloe vera), A. indica*and *Vitex negundo* to cure antifungal infections caused by

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C Albicans, a type of pathogenic yeast that lived outside the human body. Candidiasis is the most opportunistic mycosis fungal infection caused by *Candida Albicans*, the most common species. The infections range from superficial skin to systemic disease, *C Albicans, C. glabrata,* and C. parapsilosis are part of the normal flora of humans and can be isolated from the oral cavity, vaginal, and other parts of body sites from normal healthy people. So, formulating anti-fungal lotion with the extracts of C. roseus, aloe vera, A. indica, and Vitex negundo is based on natural drugs which come up with newer, safer, and more efficient drug therapy against one of the most infectious diseases.

MATERIALS AND METHODS

Collection of Materials

The main herbal extract ingredients are an extract of Catharanthus roseus (Sadabahar), Aloe vera, Azadirachta indica (Neem), and Vitex negundo (Nirgundi) used in the formulation of Anti-fungal herbal lotion. The extracts were collected from the Ayushagro Food Ayurveda industry and the extracts were properly packed and dry.

Formulation of Anti-fungal herbal lotion

For the formulation of the Anti-fungal herbal lotion, we will use the dry gum method to formulate the emulsion. Firstly, weigh the required quantity of dry extract of Catharanthus roseus, Aloe vera, Azadirachta indica, and Vitex negundo. After that, put these weighed extracts into a mortar and mix them properly with help of a pestle. Now, we will take water with help of a pipette. Add water drop by drop and stir it continuously in one direction until the primary emulsion does not form. After that, add the required quantity of ethanol, glycerine, and coconut oil, and stir it properly until the required viscosity of the emulsion form. After the preparation, take out the prepared anti-fungal lotion from the mortar and store it in a container. Now the prepared anti-fungal herbal lotion will go for evaluation testing where we can test the physical properties such as color, odor, texture, irritancy, washability, pH, viscosity, phase separation, spread-ability, and greasiness.

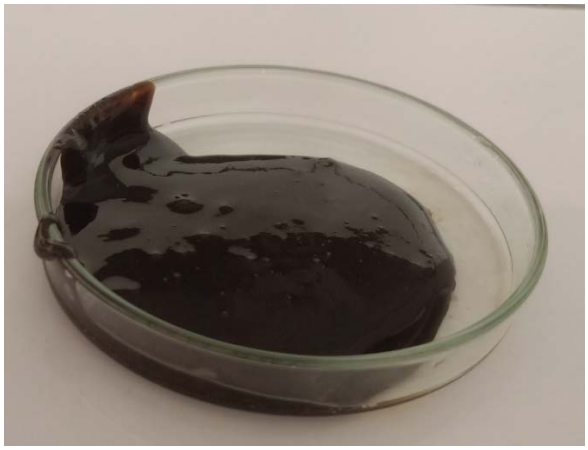

Fig. 1: Prepared Anti-fungal lotion F1

Table 1:Formula for F1 formulation of Anti-fungal Herbal Lotion

S.No.	Ingredients	Quantity
1.	Catharanthus Roseus	12gm
2.	Aloe-vera	6gm
3.	Azadirachta Indica	3gm
4.	Vitex Negundo	2gm

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5.	Ethanol	4ml
6.	Glycerine	4ml
7.	Coconut oil	1ml
8.	Distilled Water	q.s.

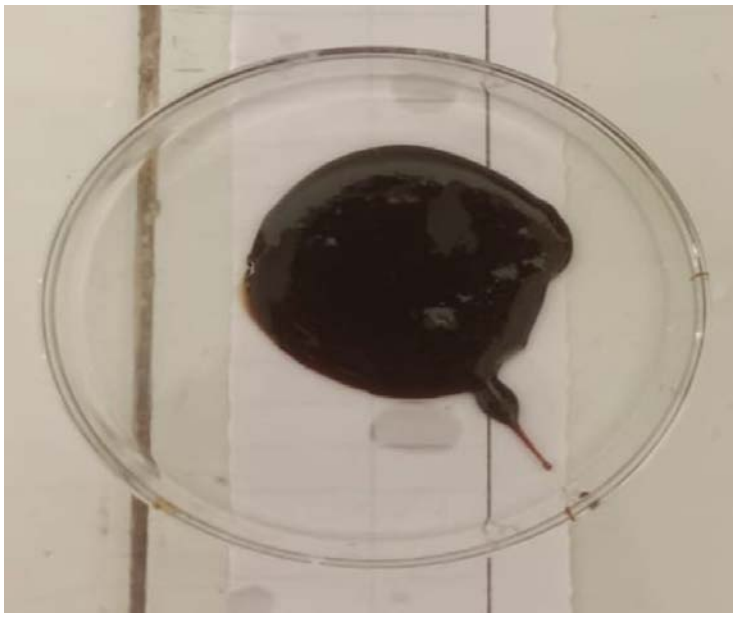

Fig. 2: Prepared Anti-fungal lotion F2

Table 2: Formula for F2 formulation of Anti-fungal Herbal Lotion

S.No.	Ingredients	Quantity
1.	Catharanthus Roseus	14gm
2.	Aloe-vera	4gm
3.	Azadirachta Indica	2gm
4.	Vitex Negundo	1gm
5.	Ethanol	6ml
6.	Glycerine	4ml
7.	Coconut oil	2ml
8.	Distilled Water	q.s.

Fig. 3: Prepared Anti-fungal lotion F3

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Table 3: Formula for F3 formulation of Anti-fungal Herbal Lotion

S.No.	Ingredients	Quantity
1.	Catharanthus Roseus	10gm
2.	Aloe-vera	4gm
3.	Azadirachta Indica	2.4gm
4.	Vitex Negundo	1.6gm
5.	Ethanol	6ml
6.	Glycerine	4ml
7.	Coconut oil	2ml
8.	Distilled Water	q.s.

Antifungal Activity

The prepared three formulations F1, F2, and F3 undergo an antifungal activity test by using zone inhibition methods. The antifungal activity is tested on three different microorganisms, Candida albicans, Candida tropicalis, and Candida parapsilosis.

Materials Required

1) Mueller Hinton Agar (MHA) Plates
2) Sabouraud Dextrose Agar (SDA) Plates
3) Fungal cultures (Candida albicans, Candida tropicalis, and Candida parapsilosis)
4) Solvent (vehicle control)
5) Whatman No 1 filter paper discs (5mm)
6) Fluconazole
7) Amount Loaded – (0 to 5000 µg/well)

Zone Inhibition Test

The Anti-fungal activity was checked by following Zone Inhibition Method. The MHA plates were inoculated by spreading with 100 µl of fungal culture, all three microorganisms (adjusted to 0.5 McFarland Unit), and followed by making the wells containing 10 µl of different concentrations (0 to 5000 µg/ml). One well in each plate was loaded with solvent alone which served as vehicle control and fluconazole disc (3 mg) was taken as the positive control. The plates of Candida tropicalis were incubated at 35.4 °C for 48 to 47 hr. The plates of Candida parapsilosis were incubated at 35.4 °C for 48 to 47 hr. The plates of Candida albicans were incubated at 30 °C for 24 h.

RESULT AND DISCUSSION

Antifungal Activity

Zone Inhibition test was performed on all three formulations (F1, F2 & F3) by using three different fungal Candida albicans, Candida tropicalis, and Candida parapsilosis. Fluconazole is used as a standard antifungal agent at the control concentration of 3000µg. On the other hand, three different formulations are used for the zone inhibition at different concentrations like 100µg, 500µg, 1000µg, 2500µg, and 5000µg for all the fungal strains.

In the results, we observed that formulation F3 shows antifungal activity on Candida tropicalis whereas there is no antifungal activity found on Candida albicans and Candida parapsilosis. The formulation F3 shows higher antifungal activity at the concentration of 5000µg against Candida tropicalis. None of the formulations show antifungal activity against Candida parapsilosis and Candida albicans at any concentration.

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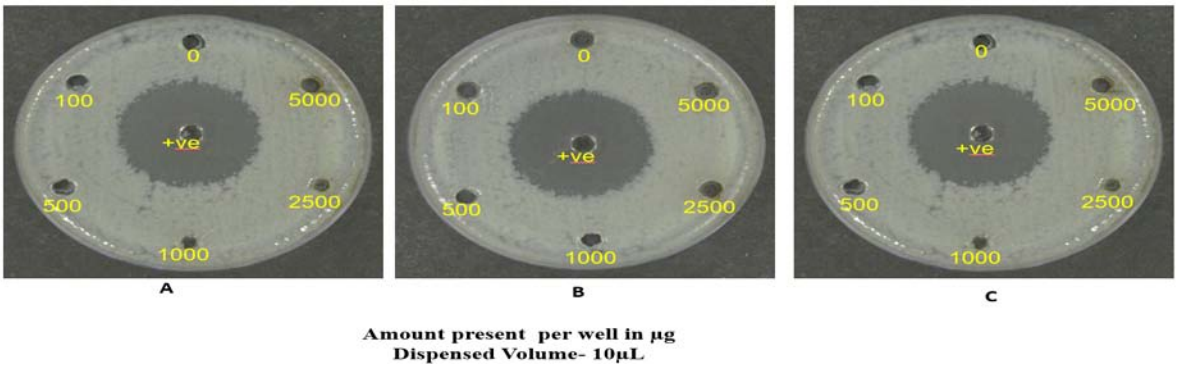

Fig.4: Antifungal Zone Inhibition Test on Candida albicans by using F1

Table 4: Zone Inhibition (in mm) by F1 against Candida albicans

Amount (µg/disk)	Plate A	Plate B	Plate C	Average	SD	SEM
Control (3000µg)	34	34	35	34.333	0.57735	0.3333
0µg	0	0	0	0	0	0
100µg	0	0	0	0	0	0
500µg	0	0	0	0	0	0
1000µg	0	0	0	0	0	0
2500µg	0	0	0	0	0	0
5000µg	0	0	0	0	0	0

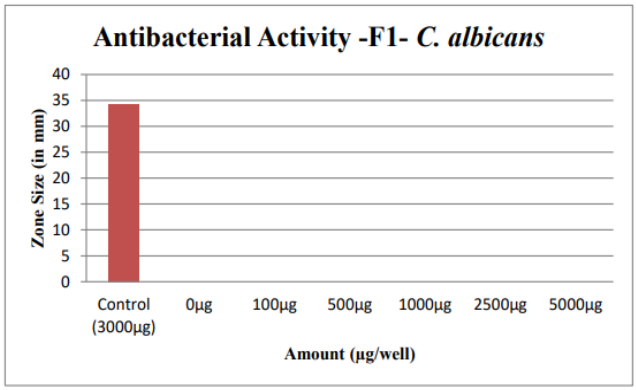

Fig.6: Graph representation of Zone inhibition by F1 against Candida albicans

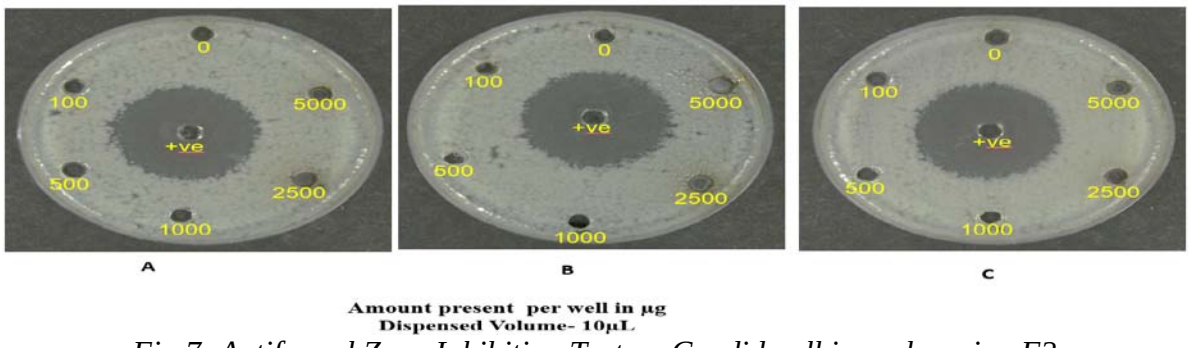

Fig.7: Antifungal Zone Inhibition Test on Candida albicans by using F2

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Table 5: Zone Inhibition (in mm) by F2 against Candida albicans

Amount (µg/disk)	Plate A	Plate B	Average	SD	SEM
Control (3000µg)	34	35	34	0.70711	0.40825
0µg	0	0	0	0	0
100µg	0	0	0	0	0
500µg	0	0	0	0	0
1000µg	0	0	0	0	0
2500µg	0	0	0	0	0
5000µg	0	0	0	0	0

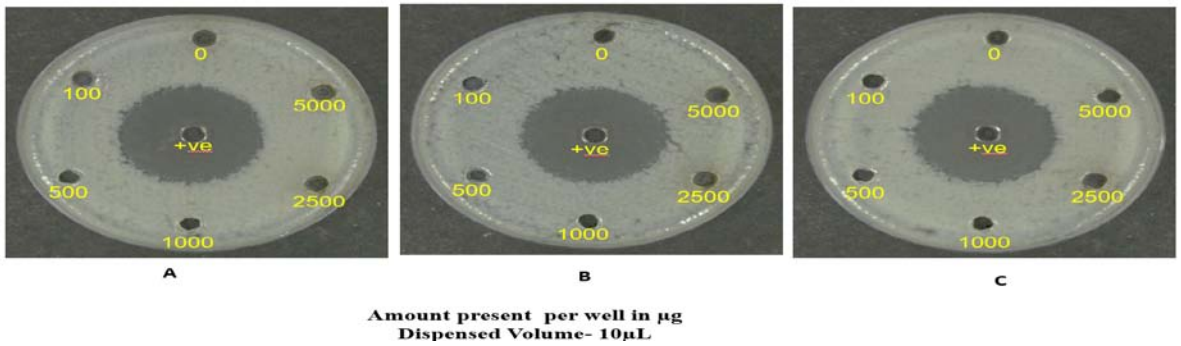

Fig.8: Antifungal Zone Inhibition Test on Candida albicans by using F3

Table 6: Zone Inhibition (in mm) by F3 against Candida albicans

Amount (µg/disk)	Plate A	Plate B	Plate C	Average	SD	SEM
Control (3000µg)	34	35	34	34.3333	0.57735	0.3333
0µg	0	0	0	0	0	0
100µg	0	0	0	0	0	0
500µg	0	0	0	0	0	0
1000µg	1	2	2	1.666	0.57735	0.3333
2500µg	6	5	5	5.3333	0.57735	0.3333
5000µg	10	11	10	10.3333	0.57735	0.3333

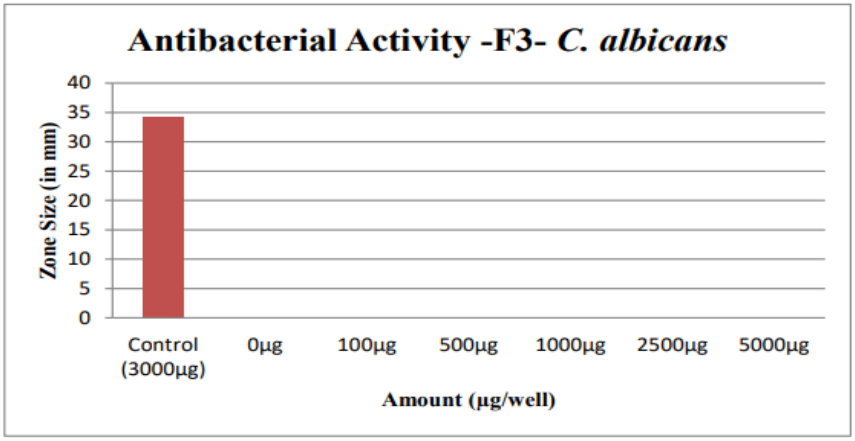

Fig.9: Graph representation of Zone inhibition by F3 against Candida albicans

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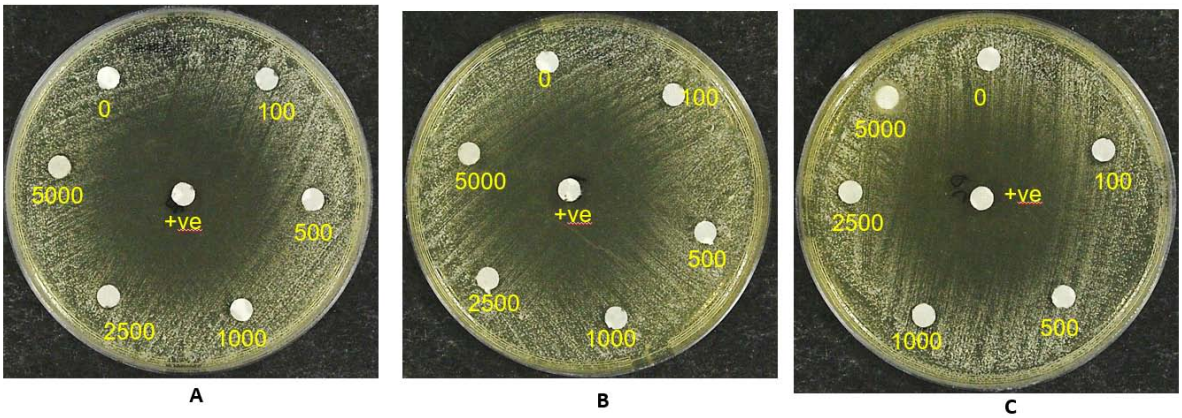

Amount present per well in µg
Dispensed Volume- 10µL

Fig.10: Antifungal Zone Inhibition Test on Candida parapsilosis by using F1

Amount (µg/disk)	Plate A	Plate B	Plate C	Average	SD	SEM
Control (3000µg)	41	46	43	43.333	2.5166	1.4529
0µg	0	0	0	0	0	0
100µg	0	0	0	0	0	0
500µg	0	0	0	0	0	0
1000µg	0	0	0	0	0	0
2500µg	0	0	0	0	0	0
5000µg	0	0	0	0	0	0

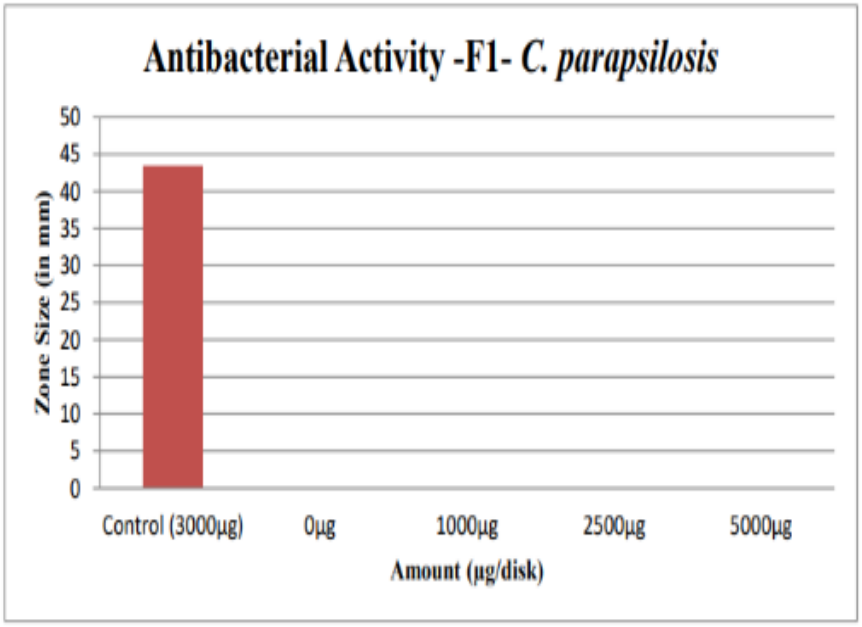

Fig.11: Graph representation of Zone inhibition by F1 against Candida parapsilosis

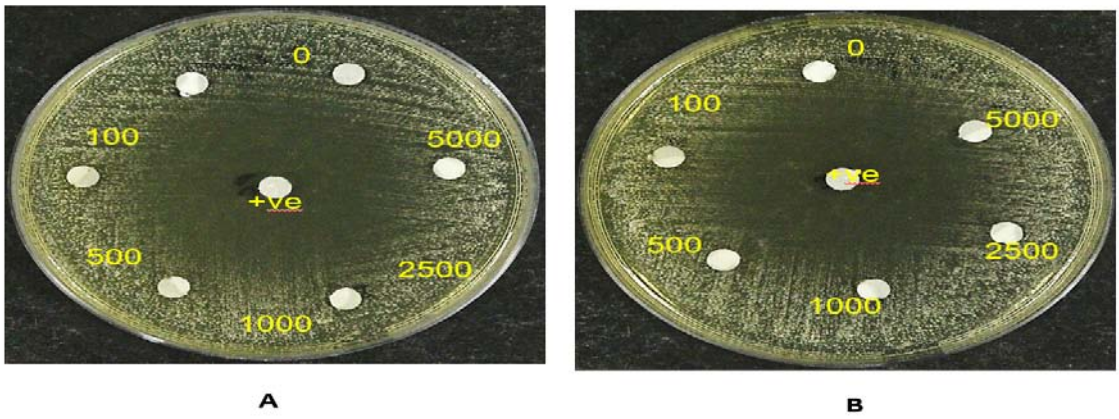

Fig.12: Antifungal Zone Inhibition Test on Candida parapsilosis by using F2

Table 8: Zone Inhibition (in mm) by F2 against Candida parapsilosis

Amount (µg/disk)	Plate A	Plate B	Average	SD	SEM
Control (3000µg)	45	45	45	0	0
0µg	0	0	0	0	0
100µg	0	0	0	0	0
500µg	0	0	0	0	0
1000µg	0	0	0	0	0
2500µg	0	0	0	0	0
5000µg	0	0	0	0	0

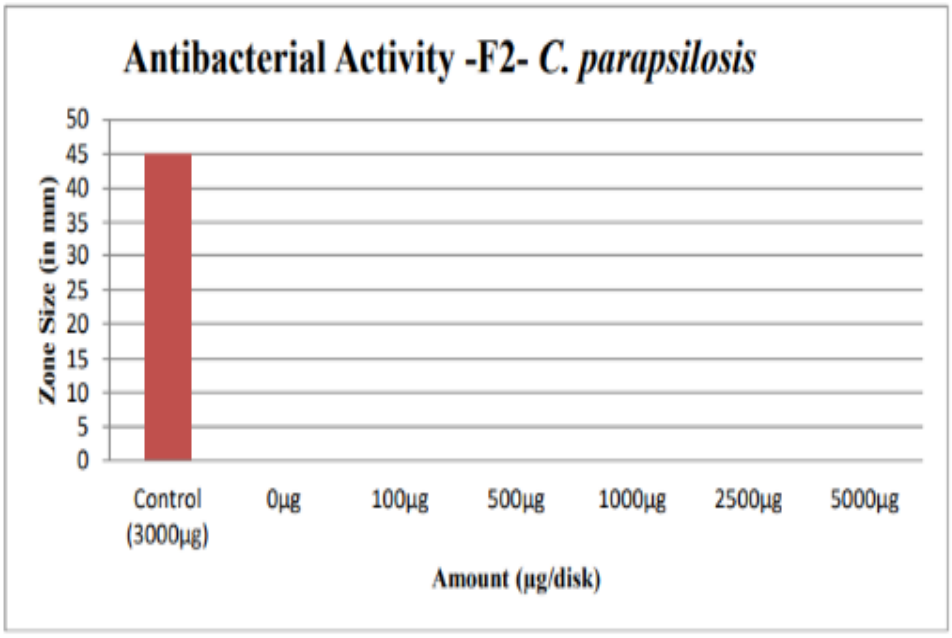

Fig.13: Graph representation of Zone inhibition by F2 against Candida parapsilosis

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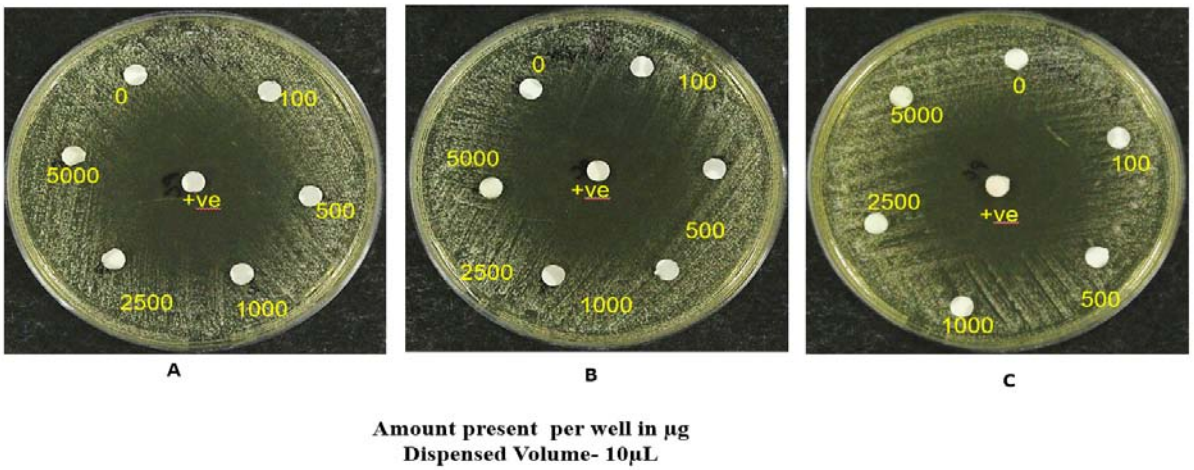

Fig.14: Antifungal Zone Inhibition Test on Candida parapsilosis by using F3

Table 9: Zone Inhibition (in mm) by F3 against Candida parapsilosis

Amount (µg/disk)	Plate A	Plate B	Plate C	Average	SD	SEM
Control (3000µg)	46	45	46	45.666	0.57735	0.3333
0µg	0	0	0	0	0	0
100µg	0	0	0	0	0	0
500µg	0	0	0	0	0	0
1000µg	0	0	0	0	0	0
2500µg	0	0	0	0	0	0
5000µg	0	0	0	0	0	0

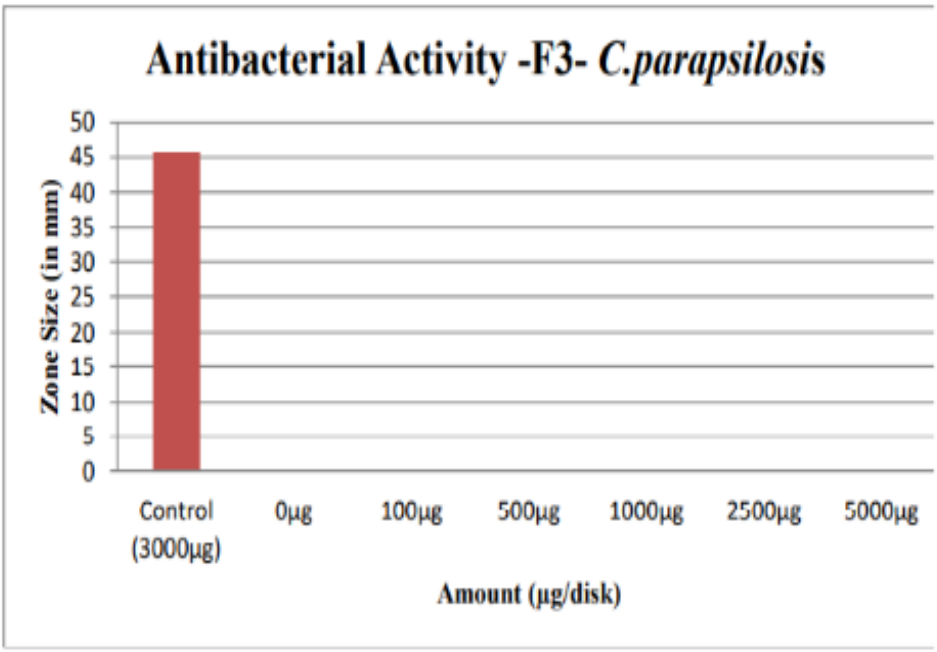

Fig.15: Graph representation of Zone inhibition by F3 against Candida parapsilosis

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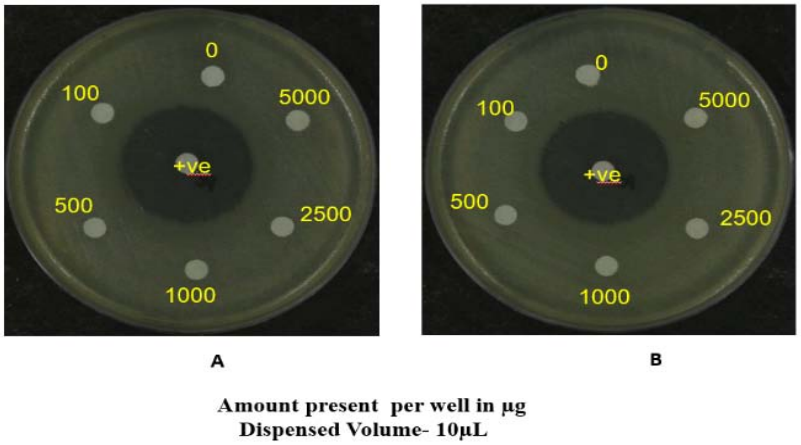


Fig.16: Antifungal Zone Inhibition Test on Candida tropicalis by using F1

Table 10: Zone Inhibition (in mm) by F1 against Candida tropicalis

Amount (µg/disk)	Plate A	Plate B	Average	SD	SEM
Control (3000µg)	38	37	37.5	0.70711	0.40825
0µg	0	0	0	0	0
100µg	0	0	0	0	0
500µg	0	0	0	0	0
1000µg	0	0	0	0	0
2500µg	0	0	0	0	0
5000µg	0	0	0	0	0

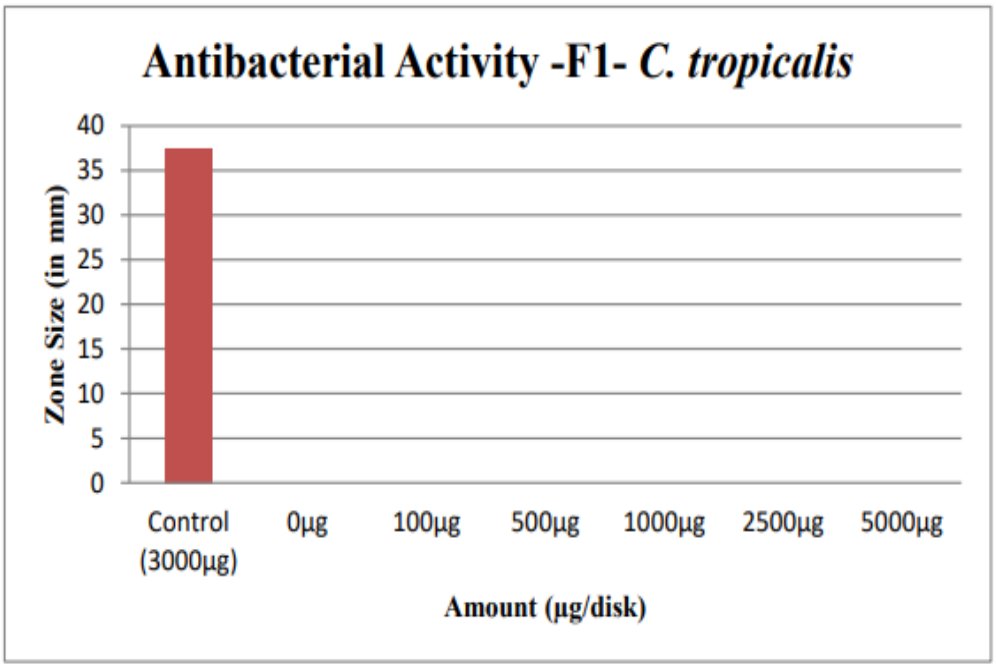


Fig.17: Graph representation of Zone inhibition by F1 against Candida tropicalis

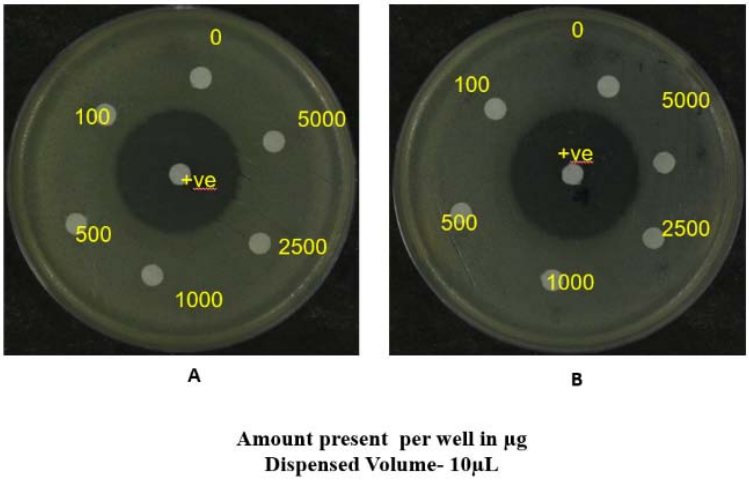
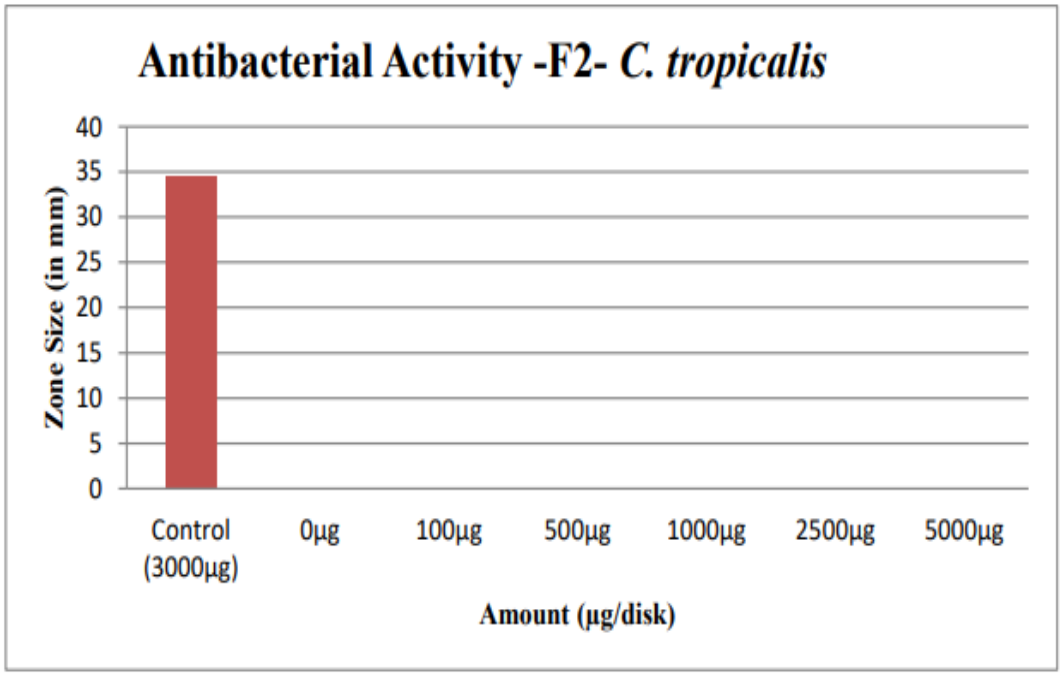


Fig.18: Antifungal Zone Inhibition Test on Candida tropicalis by using F2

Table 11: Zone Inhibition (in mm) by F2 against Candida tropicalis

Amount (µg/disk)	Plate A	Plate B	Average	SD	SEM
Control (3000µg)	35	34	34.5	0.70711	0.40825
0µg	0	0	0	0	0
100µg	0	0	0	0	0
500µg	0	0	0	0	0
1000µg	0	0	0	0	0
2500µg	0	0	0	0	0
5000µg	0	0	0	0	0



Fige19: Graph representation of Zone inhibition by F2 against Candida tropicalis

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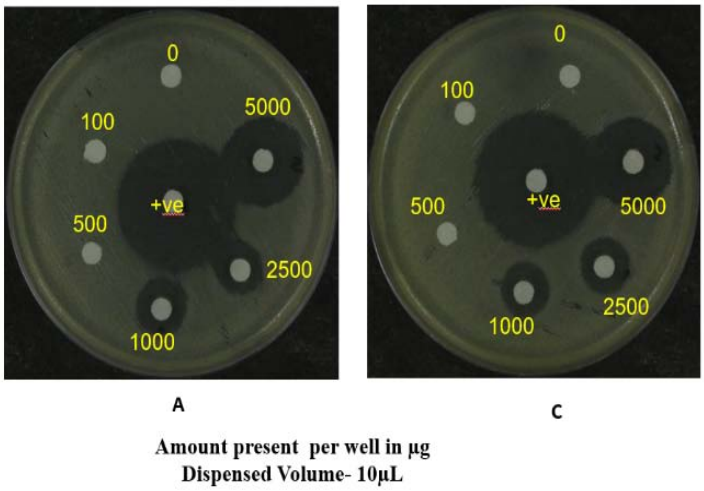

Fig.20: Antifungal Zone Inhibition Test on Candida tropicalis by using F3

Table 12: Zone Inhibition (in mm) by F3 against *Candida tropicalis*

Amount (µg/disk)	Plate A	Plate B	Average	SD	SEM
Control (3000µg)	32	32	32	1.5275	0.8819
0µg	0	0	0	0	0
100µg	0	0	0	0	0
500µg	0	0	0	0	0
1000µg	13	13	13	0	0
2500µg	14	14	14	0	0
5000µg	21	21	21	0	0

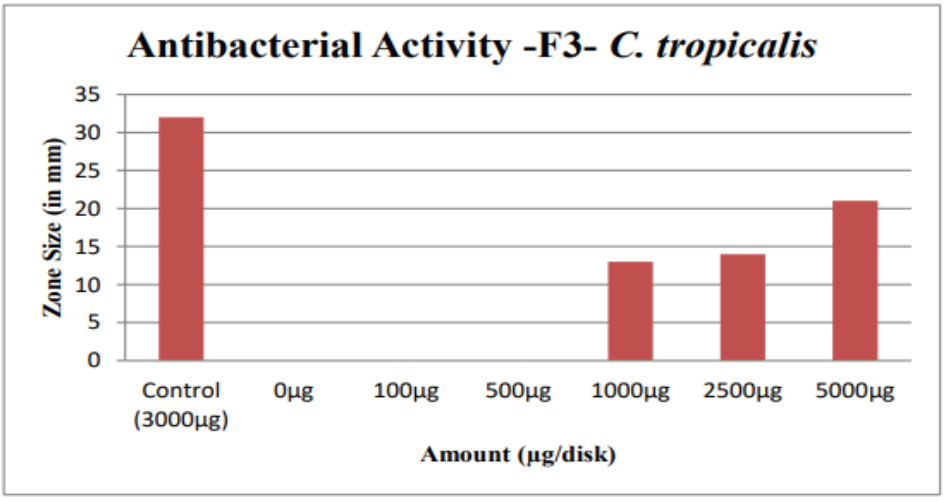

Fig. 21: Graph representation of Zone inhibition by F3 against Candida tropicalis

Evaluation of formulated Anti-fungal Herbal Lotion

- **Physical Evaluation**

In this test, we observed the color, odor, texture, and state of the prepared formulations F1 & F2 of the anti-fungal herbal lotion.

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Table 13: Physical evaluation of the formulation (F1, F2 &F3)

S.No.	Parameters	Observation
1.	Color	Light brown
2.	Odor	Pleasant
3.	Texture	Smooth
4.	State	Semi-solid

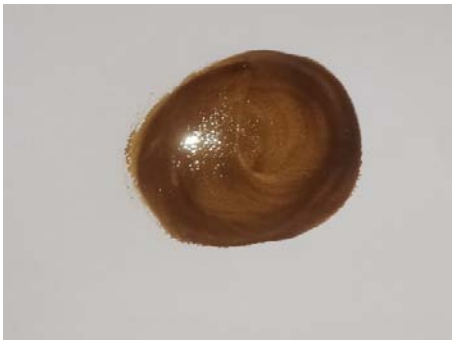

Fig. 22: Color of the F1 formulation

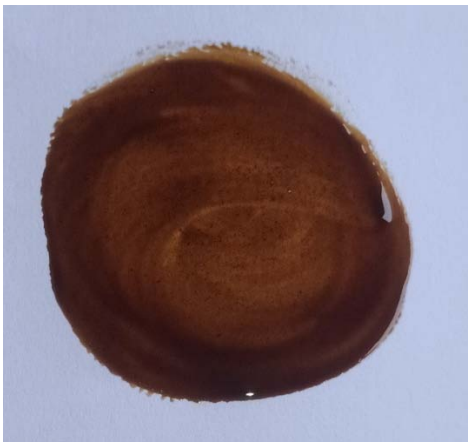

Fig. 23: Color of the F2 formulation

Fig. 24: Color of the F3 formulation

- **pH**

The pH value of the F1, F2& F3 formulation of the anti-fungal lotion was measured at 25°C by using a pH meter (Systronics digital pH meter 335, India).

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Table 14: pH observation of the F1, F2 & F3 formulation

S.No.	Formulation	pH
1.	F1	6.7
2.	F2	6.8
3.	F3	7

- **Phase Separation**

The prepared formulations F1, F2& F3 of the anti-fungal herbal lotion are kept in a closed container at the temperature of 20 degrees Celsius to 100 degrees Celsius for 24 hours for 20 days. According to the results, there were no phase separations observed in the prepared F1, F2& F3 anti-fungal herbal formulations.

Table 15: Phase separation observation of the F1, F2 & F3 formulation

S.No.	Formulation	Phase separation
1.	F1	No phase separation
2.	F2	No phase separation
3.	F3	No phase separation

- **Irritancy**

Marked a certain area on the dorsal surface of the hand and apply the formulations F1, F2& F3, and left it for 24 hours. Then checked for irritancy, erythema, and edema. According to the results, there was no irritancy observed by the use of prepared anti-fungal herbal lotion in both formulations F1, F2& F3.

Table 16: Irritancy observation of the F1, F2 & F3 formulation

S.No.	Formulation	Irritancy	Erythema	Edema
1.	F1	Nil	Nil	Nil
2.	F2	Nil	Nil	Nil
3.	F3	Nil	Nil	Nil

- **Washability**

Apply a certain amount of the F1, F2& F3 formulations of anti-fungal herbal lotion on the surface of the hand and then wash it with tap water. According to the results, the prepared formulations F1, F2& F3 of anti-fungal herbal lotion were easily washable.

Table 17: Washability observation of the F1, F2 & F3 formulation

S.No.	Formulation	Washability
1.	F1	Washable
2.	F2	Washable
3.	F3	Washable

- **Viscosity**

By the use of the Brooke field viscometer, we can test the viscosity of the prepared anti-fungal herbal lotion formulation F1, F2& F3 at a room temperature of 25 degrees Celsius using spindle no. 63 at 2.5 RPM. According to the results, the prepared formulations F1, F2& F3 showed significant viscosity

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S.No.	Formulation	Viscosity (Cps)
1.	F1	35880
2.	F2	32021
3.	F3	39081

- **Greasiness**

The prepared formulations F1, F2& F3 of anti-fungal herbal lotion were applied on the surface of the hand in the form of a patch and checked. According to the results, both F1, F2& F3 formulations of anti-fungal herbal lotion were non-greasy.

Table 19: Greasiness observation of the F1, F2 & F3 formulation

S.No.	Formulation	Greasiness
1.	F1	Non-Greasy
2.	F2	Non-Greasy
3.	F3	Non-Greasy

- **Spread-ability**

In this test, formulation F3 took lesser time for the separation of the two slides than the F1 & F2formulations. It means the F3 formulation has better spread-ability than F1 & F2. So according to the results, the prepared formulation F3 has better spread-ability properties.

Table 20: Spread-ability observation of the F1, F2 & F3 formulation

S.No.	Formulation	Spread-ability (g×cm/sec)
1.	F1	46.8
2.	F2	45.18
3.	F3	47

CONCLUSION

In the present study, we try to make Antifungal lotion by the use of herbal medicinal plants like Catharanthus roseus, Aloe vera, Azadirachta indica, and Vitex negundo.

We deeply study many articles to understand the properties of these herbal medicinal plants. We study the active components of the Catharanthus roseus with their properties and also tried to classify their clinical efficacy to understand the major role of the Catharanthus roseus in the medicinal field. Catharanthus roseus is most effective in fungal and microbial skin diseases. In a study, we observed antifungal activity against Candida albicans.

Aloe vera and Azadirachta indica were used as antimicrobial and antibacterial agents from ancient times. In our study, we found the same result for Aloe vera and Azadirachta indica. The aloe vera gel is most effective against fungal infection. In a study, we observed aloe vera gel show 99.33% high zone inhibition against Candida albicans whereas aloe vera leaves did not show any fungal activities against Candida albicans. Vitex negundo also has some antifungal activity against Candida albicans.

In this study, we use all these high-class medicinal plants to treat fungal infections by formulating them in lotion form. We prepared three different formulations F1, F2, and F3

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with different quantities of different medicinal plants. All these medicinal plants are compatible with each other.

Formulations F3 show good zone inhibition results against *Candida tropicalis*. At the concentration of 1000µg, 2500µg, and 5000µg.

In the antifungal activity test, we found some good results against *Candida tropicalis*. We did not find any antifungal activity in any of these formulations against *Candida albicans* and *Candida parapsilosis*. The formulation F3 shows higher antifungal activity at the concentration of 5000µg against *Candida tropicalis*. So, the final result is impressive and further study makes this lotion much better for future clinical use.

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