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MICROBIOLOGICAL EVALUATION OF *Crossandra* infundibuliformis AGAINST Candida albicans: An *In-vitro* ANALYSIS

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ABSTRACT:

Crossandra infundibuliformis, a firecracker flower native to southern India and Sri Lanka, is often used as an ornamental plant for its medicinal properties. Traditional medicine often involves various remedies using different parts of a plant, with ethnomedicine playing a role in drug discovery. Infectious diseases remain a significant cause of morbidity and mortality among humans, especially in developing countries. In recent years, resistance to antibiotics by microbes has increased, forcing scientists to search for new antimicrobial substances. Plant- based antimicrobials represent a vast untapped source of medicines. Over the last few decades, the incidence of opportunistic infections in patients treated with immunosuppressive drugs or intensive chemotherapy has increased at an alarming rate. Higher plants produce diverse chemical compounds with different biological activities, which have an important ecological role. Plant extracts have been used as alternative sources for treating fungal infections, such as *Candida albicans*, which are responsible for 80-90% of infections in dermatology. The phytochemical test of *Crossandra infundibuliformis* leaf extract revealed its antibacterial, antifungal, and anticandidal activity. By testing the inhibitory level against *Candida albicans* species, a topical substance could be developed against these infections. Antifungal creams, liquids, or sprays have shown effective antifungal activity in the past few years. Herbal extracts and essential oils have shown effective antifungal activity, and nanohydrogels can be applied to infected areas to reduce fungal infections. Skin acts as a protective barrier against external agents, sunburn, and body temperature, but pathogens can cause skin diseases or infections. Fungal infections are more severe because they occur on the third layer of the skin, leading to subcutaneous infections. In this study, the extract from *Crossandra infundibuliformis* will be collected and formulated as a cream against *Candida albicans*, potentially helping to treat skininfections caused by *Candida albicans.*

KEYWORDS: *Crossandra infundibuliformis, Candida albicans and* anticandidal cream

INTRODUCTION

Crossandra infundibuliformis, the firecracker flower, is a species of flowering plant in the family Acanthaceae, native to southern India and Sri Lanka. It is most often found in south Indian region Andhra Pradesh, Tamil Nadu,and Kerala. It often used as an ornamental plant, is marketed as such. The species is often grown to beautify kitchen gardens as it is small in size and sports attractive flowers. Thus, we are excited to learn from literature and also from folk, the medicinal uses of *C. infundibuliformis*. Due to its medicinal value, the various parts of thisplant are used to treat many disease.

Traditional medicine is usually associated with various remedies using different parts of a plant. Plants used in traditional medicine are more likely to have pharmacologically active compounds. Therefore, ethnomedicine plays a role in the drug discovery. Various drugs used in today's medicines are derived from plants (Eg: morphine, codeine, salicin). Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. Even though pharmaceutical companies have produced a number of new antibacterial drugs in the last few years, resistance to these drugs by microbes have increased and has now become a global concern. This situation forced scientists to search for new antimicrobial substances. Hence much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. Plant-based antimicrobials represent a vast untapped source of medicines

Over the last few decades, the incidence of opportunistic infections in patients treated with immunosuppressive drugs or intensive chemotherapy is increasing at an alarming rate. Resistance to antibiotics is a major problem in the management of infections caused by microbes. Several new strategies to control such infections have been considered in recent years. These include the use of antibiotic combinations, the development of new members of existing antibiotic classes and the introduction of novel agents. Higher plants, which are able to do photosynthesis, produce hundreds to thousands of diverse chemical compounds with different biological activities. It is believed that these compounds have an important ecological role. They can work as pollinator attractants and as chemical defences against insects, herbivores and microorganisms.

These antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation of those extracts that yielded active principles. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries.

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against micro-organism infections. Nowadays the chance of outbreak of opportunistic microbes are high due to the adverse changes in the health of humans. The opportunistic microbes such as Candida species are the largest genus of medically important yeast. The species of Candida such as Candida albicansare said to be pathogenic and they are also the normal flora of gut microbes. It causes candidiasis which affects the areas of skin, genitals, mouth, throat, blood. These infections are often cured by the antifungal and anti-candidal agents. But here a question arises, if all the candida species eventually became resistance to the antifungals or anticandidals what can be the alternative treatments can be provided. So to this arising question we make use of the natural plant extracts as an alternative sources for such infections like the ancient period.

Candida spp. infections are one of the most common fungal infections in dermatology. *Candida albicans* is responsible for 80–90% of infections, but other *Candida* species are frequently seen as causative pathogen *Candida* infections are considered opportunistic in most cases because *Candida albicans* is a normally commensal fungus. However, when host-immunity is impaired for various possible reasons, a pathogenic infection may occur. Overall, a balance between the host defence system and the virulence factors of *Candida albicans* is the key to the commensal relationship. *Candida albicans* usually causes superficial skin infections, while "deep" mycoses, with the dermis and subcutis' involvement due to *Candida*, are rare. However, in severely immunocompromised patients, an invasive fungal infection may occur, resulting in deep penetration and systemic candidiasis, often with a fatal outcome.

The phytochemical test of leaf extract of *Crossandra infundibuliformis* described that it contains the activity of antibacterial, antifungal and anticandidal activity. By using the leaf extract we are going to test the inhibitory level against the *Candida albicans* species so that we could come up with product such as topical substance against the infections of *Candida albicans* .We can able to test the anticandidal activity of the leaf extract by using minimal inhibitory concentration .The minimal inhibitory concentration test are the lowest concentration of the antimicrobial substance that can inhibit the visible growth of the microorganisms. With the help of performing this test we could easily find the antimicrobial activity of the leaf extract of the plant.

Human skin acts as a physical barrier; however, sometimes the skin gets infected by fungi, which becomes more severe if the infection occurs on the third layer of the skin. antifungal creams, liquids, or sprays are available to treat fungal infections. Over the past few years, herbal extracts and various essential oils have shown effective antifungal activity. These gels protect plant-based bioactive compounds and are effective delivery agents because they release multiple bioactive compounds in the targeted area. Nanohydrogels can be applied to infected areas, and due to their contagious nature and penetration power, they get directly absorbed through the skin, quickly reaching the skin's third layer and effectively reducing thefungal infection.

Skin acts as a protector of the internal organs by shielding against external agents, sunburn, and by regulating body temperature; however, sometimes pathogens invade the body and disturb the skin protective properties, leading to skin diseases or infections . Bacteria, viruses, parasites, and fungi can cause skin diseases. Fungal infections are more severe because they occur on the third layer of the skin . Fungi act on keratin tissue such as skin, nails, and hair . In the skin, fungi lead to subcutaneous infections, and over the past years, the cases of fungal skin infections have been increasing rapidly, especially in immune-compromised individuals.

In this study we are going to collect extract from Crossandra infundibuliformis and formulate a cream against Candida albicans. It will be useful in the treatment of Candida albicans causing skin infection.

2. MATERIALS AND METHODSCOLLECTION OF SAMPLE

Crossandra infundibuliformis plant where collected and authenticated by Tamil NaduAgriculture university, Coimbatore Tamil Nadu .

COLLECTION FUNGAL SPECIMEN

Candida albicans culture was obtained from Kovai Medical Center and Hospital,Coimbatore and microscopic observation has been done .

EXTRACTION PROCESS

Leaves of *Crossandra infundibuliformis* was sun dried and powdered . The air- dried leaves of *Crossandra infundibuliformis* where extracted with ethyl acetate in Soxhlet apparatus for 15-20 hrs using 200ml of solvent.

CONCENTRATION OF SAMPLE

The sample was concentrated using rotary evaporator at 75°C for 30 min and stored at 4°C for further use.

PHYTOCHEMICAL ANALYSIS

Tannin:

To 2ml of plant extract boiled with the 10ml of distilled water and 0.1% ferric chloride was added. Presence of blue-black colour indicates the positive result.

Saponin:

To 0.5ml of extract and 5ml of distilled water was combined and agitated. Foam formation indicates the positive result.

Terpenoids:

To 1ml of extract was combined with 4ml of chloroform and 6ml of sulphuric acid.

Presence of Reddish brown colour indicates the positive result.

Alkaloids:

Few drops of Mayer's reagent were added to 1 mL of extract. A yellowish or white precipitate was formed, indicating the presence of alkaloids.

Anthroquinone:

One drop of concentrated ammonium hydroxide was added to 10 mg of each extract, previously dissolved in isopropyl alcohol. After two minutes, formation of red color indicated the presence of anthraquinone.

Phytosterols:

Plant extracts were filtered after treating with chloroform. The filtrate obtained was treated with few drops acetic anhydride, boiled and cooled at room temperature. Conc. H_2SO_4 was added and formation of brown-color ring indicates the positive result.

Proteins and Amino acid:

About 0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent were added and heated. The appearance of pink or purple colour indicates the presenceof proteins and amino acids.

Carbohydrates:

To 5 mL of Benedict's reagent, 8-10 drops extract were added, then heated for five minutes; the presence of dark red precipitate indicates the presence of carbohydrates.

Steroids:

The test extract was shaken with chloroform and concentrated H_2SO_4 was added along the walls of a test tube, a red colour indicates the presence of steroids.

Phenols:

To 2ml of plant extract, 2ml of distilled water followed by 10 % $FeCl_3$ solution was added. Bluish black colour indicates the presence of phenol.

Glycosides:

To 0.5ml of extract was dissolved in 1ml of water and then aqueo NAOH solution was added. Formation of yellow colour indicates the presence of glycosides.

ANTICANDIDIAL ACTIVITY

The extracts were evaluated for their in vitro anticandidal activity against pathogenic fungi using agar diffusion method with Sabouraud's dextrose agar (Hi-Media). Suspension of fungi were prepared and applied to agar plates with serially diluted compounds to be tested. Amphotericin B was used as a reference standard. The plates were incubated at 26°C for 72 hour's.

MINIMAL INHIBITORY CONCENTRATION

After the Dilution preparation and method selection, the extracts and their various concentrations are analysed to check the Minimal inhibitory concentration (MIC) of the lowest amount of Extracts that inhibit the growth of selected bacteria. When the extracts are soaked in a plane disc and Placed in a petri plate along with the selected Bacteria, the extracts producethe zone of inhibition around each well of antibiotics.

FORMULATION OF CREAM

1. Preparation of oil phase

Coconut oil , petroleum jelly , white bees wax , paraffin wax were mixed together in a porcelain dish at 75°C

2. Preparation of aqueous phase

Extract of *Crossandra infundibuliformis* , water and preservative were taken into another porcelain dish and heated this mixture at 75°C

3. Addition of aqueous phase and oil phase

At 75°C, the aqueous phase was added to the oil phase by stirring continuously . After the transfer was complete it was allowed to cool to room temperature while being stirred constantly . Just before the finished product was moved to suitable container , the perfume was added last. The cream was the evaluated for a various physical characteristics.

Table 1: formulation of cream

S.NO	INGREDIENTS	QUANTITY
1.	*Crossandra infundibuliformis*	2gm
2.	Coconut oil	19ml
3.	Petroleum jelly	4gm
4.	White bees wax	7.5gm
5.	Paraffin wax	0.5gm
6	Borax	0.5gm
7.	Water	17.5ml
8.	Eucalyptus oil	2-3 drops

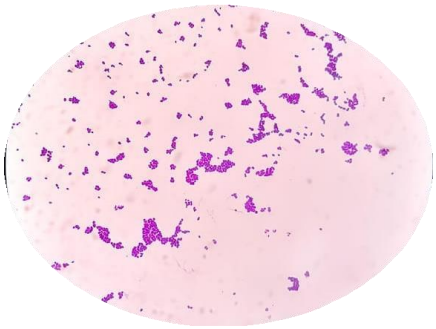
PARAMETERS FOR CREAM

Physical appearance - All antifungal herbal creams physical appearance was analysedvisually and result was tabulated below.

Determination of pH - pH of formulation cream was measured by using pH meter.

RESULTS AND DISCUSSION:

Figure 1 : Microscopic observation of *Candida albicans*



Candida albicans exhibit spherical to subspherical large yeast-like cells with budding,blastoconidia, and pseudohyphae

Figure 2: Soxhlet extraction

Figure 3 : Rotary evaporation

Leaf has been extracted using Soxhlet apparatus by solvent ethyl acetate

Figure 4: Phytochemical analysis .

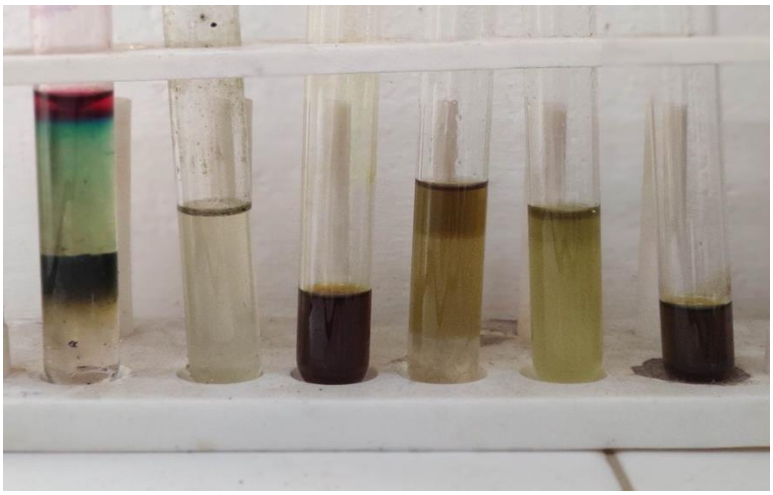
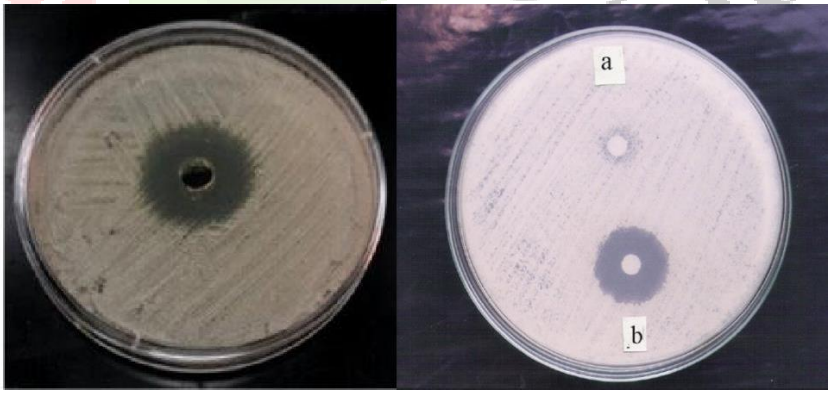


Table 2: Phytochemical analysis

S.NO	TEST	RESULT
1.	Alkaloids	+
2.	Saponins	+
3.	Phenolics & Tannins	+
4.	Flavonoids	+
5.	Carbohydrates	-
6.	Phytosterols	+
7.	Terpenoids	-
8.	Oils & fats	-

Figure 5: Antimicrobial activity



The result showed that leaf extract can control the fungal disease caused by *Candida albicans.* Amphotericin B used as positive control and Fluconazole used as negative control.

Table 3: Antibacterial activity of the formulated cream

ANTIBIOTICS USED	ZONE OF INHIBITION (mm)	RESULTS
Fluconazole (a)	01	Sensitive
Amphotericin B (b)	18	Resistant
extract	15	Resistant

Table 4: Anticandidal activity of the formulated cream

Organism	Nc	Pc	20	40	60	80	100
Candida albicans (Pc- Amphotericin B)	0.75	0.17	0.55	0.52	0.45	0.47	**0.41**

Figure 6: Formulated anticandidal cream

Table 5: Physical parameters of formulated cream

Colour	Homogeneity	Consistency
White	Good	Good

SUMMARY AND CONCLUSION

Crossandra infundibuliformis also clearly expressed it have many pharmacological property of this plant . Due to possessing extensive pharmacological activities , the *Crossandra infundibuliformis* can be safely regarded as one of the morden catholicons . Extract of *Crossandra infundibuliformis* plant contain more bioactive principles, which act against the representative human pathogens.

Crossandra infundibuliformis could be useful as highly applied therapeutic against for a variety of disorder in the near future to cure human disease as well as some animal disease . It has anti-bacterial, anti-cancer, anti-ulcer, anti-fungal activity. It used as folklore medicines since ancient times for all it's beneficial effects . So that further studies are yet to be conducted on the entire plant to reap benefits & protect our traditional wealth of nature .

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