Antifungal Activity of *Azadirachta Indica* and *Antigonon Leptopus Leaves* Extracts against *Candida albicans*

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Abstract*— Candidiasis is an opportunistic infection caused by yeast of the genus Candida, primarily Candida albicans, which is the predominant species in fungal infections that can damage the skin and mucosa in humans. Phytoconstituents found in plants has comparable efficacy to synthetic medications in combating harmful microorganisms. The current in-vitro evaluation study was conducted to investigate the antifungal activity of combination of Azadirachta indica and Antigonon leptopus leaves extracts. Antifungal activity of ethanolic extract of leaves of Azadirachta indica and Antigonon leptopus against Candida albicans was carried out utilising agar well diffusion method followed by broth microdilution method using 96 well microtitre plates as per CLSI guidelines. Fluconazole was used a standard in the study. The results showed that the fungal growth was inhibited at the concentrations of 500 µg/mL for the Azadirachta indica extract and 1000 µg/mL for the Antigonon leptopus leaves extracts. Analysis of Azadirachta indica leaves and Antigonon leptopus extracts has been reported for the presence of wide range of phytoconstituents, which are recognised exclusively for their antimicrobial effects. The investigation resulted in a new study on the therapeutic benefits of combination of Azadirachta indica leaves extract with Antigonon leptopus leaves extracts to cure fungal infections. Both leaves extracts have shown improved and broad antifungal properties, making them good choices for treating candidiasis when used in suitable formulations.

Index Terms- Azadirachta indica;* Antigonon leptopus extract; *Antifungal; Candida albicans, Minimum inhibitory concentration

I. INTRODUCTION

Candidiasis is an opportunistic infection caused by yeast of the genus Candida, primarily C. albicans that can damage the skin and mucosa in humans. The use of corticosteroids, parenteral nutrition, immunosuppression, antibiotic treatment with broad spectrum drugs, and exposure to invasive medical procedures like haemodialysis, abdominal surgery, and intravascular catheter insertion are risk factors for the increasing frequency of infections.[1]. Although azoles like fluconazole are still used extensively as antifungal medications, the development of resistant strains has resulted from their overuse in clinical settings. Medicinal plants, on the other hand, continue to be an invaluable source of safe, less toxic, cheaper, readily available, and dependable drug resources on a global scale.

The growing resistance of C. albicans to these antifungal compounds and the limited availability of medications have prompted the exploration of new therapeutic options derived from plants and their essential oils, which have been traditionally employed for their antifungal qualities. Among the various strategies to combat drug resistance, the investigation of novel and potent natural products that exhibit anti-fungal properties against C. albicans biofilm cells, while causing minimal harm to healthy cells, is expected to have a substantial influence on the treatment and control of fungal infections associated with biofilms.[2]. The Azadirachta indica tree is a member of the Meliaceae family and is commonly found in tropical and semitropical climates. The tree is characterised by rapid growth, reaching a height of 20-23 metres. Its trunk is straight; the leaves are complex, imparipinnate, consisting of 5–15 leaflets. The fruits of this plant are green drupes that become golden yellow when they ripen. [3] while the plant Antigonon leptopus is in the family Polygonaceae. It is climbing plant with thin stems often grown in parks. The leaves grow in pairs and are cordate-ovate or triangular, whole, and pointy to sharp. This flower is very pink.

Fruits have one seed and are hard, nut-like, biconvex, and compact.

Traditionally, Antigonon leptopus have been used to treat diabetes, asthma, liver and spleen disorders, cough and throat constriction.[4] Antimicrobial effect of Azadirachta indica leaves extract have been reported to inhibit the growth of molds, yeasts and dermatophytes [5]-[10] while Antigonon leptopus leaves extract have been reported to inhibit molds, yeasts and bacteria.[4][11] The present investigation Antigonon leptopus was designed to screen antimicrobial activity of Azadirachta indica leaves extract in combination with Antigonon leptopus leaves extract against Candida albicans.

II. MATERIAL AND METHODS

The mature leaves of the Azadirachta indica and Antigonon leptopus plant were collected from the vicinity of Hindi Vishwa Vidyalaya, Wardha and the campus of Mahatma Gandhi Institute for Rural Industrialization, Wardha, Maharashtra, India respectively. Specimens of herbarium were authenticated and deposited at the Department of Botany, Bajaj Science College, Wardha, Maharashtra, India. The leaves were thoroughly cleaned, dried in a shaded area for duration of 14 days, and subsequently ground into a fine powder. 25 grams each of Azadirachta indica and Antigonon leptopus leaves powder were treated separately with 100 ml of ethanol and soaked overnight. The suspension was subjected to centrifugation at 5000 rpm for 20 minutes, followed by filtration. The liquid portion was evaporated in aseptic area in glass petri dishes under UV light. The high-performance liquid chromatography and phytochemical evaluation of Azadirachta indica and Antigonon leptopus leaves ethanolic extract were carried out, and the dried extract was stored at a temperature of -4°C. Fungal strain of Candida albicans (MTCC 227) was procured from MTCC Chandigardh, India. Saborauds Dextrose Agar, Modified Saborauds Dextrose Agar, Saborauds Dextrose broth, Potato Dextrose Agar, Dimethyl Sulfoxide (DMSO), and Fluconazole as the standard were used in the study. ANOVA was used as the statistical tool. Freeze-dried fungi were activated in appropriate growth conditions on suitable media as suggested by Microbial Type Culture Collection and Gene Bank (MTCC) Chandigardh, India. After being activated, the fungi were subcultured and incubated at about 37°C for 48 hrs. The fungal inoculums were prepared by using freshly cultured fungal broth and adjusted to a turbidity of 0.5 McFarland units. The standardization process was conducted using the UV Visible Spectrophotometer (Agilent Cary 100 made USA).

2.1 Determination of Antifungal Activity

Neem Leaves extract was diluted with Dimethyl Sulfoxide (DMSO) at a 1 g/ml concentration. The Neem leaves extract was further diluted to reach the required concentration of 50–500 µg/ml. The antifungal activity was evaluated using Saboraud's Dextrose Agar media by the agar-well diffusion method. The fungal inoculum was homogeneously distributed on agar plates using a sterilized glass spreader. Four wells, each with a diameter of 6 mm, were created on an agar plate using a sterile cork borer. The four wells were incorporated with 50 µl of Azadirachta indica extract, with 50 to 500 µg/ml concentrations. The agar plates incubated for 48 hrs at 37°C. [12] Furthermore, the antifungal activity of Antigonon leptopus leaves extract was investigated. The inhibitory zone's diameter was measured by a hi-antibiotic zone reader (Prolab, Mumbai India). Fluconazole and DMSO were used as a standard and a control, respectively. The experiment was repeated three times, and the results were recorded as the average of those three separate trials. Four combinations of Azadirachta indica leaves extract and Antigonon leptopus leaves extract were prepared based on the prior data interpretation, and a bioassay was conducted in triplicate.

2.2 Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of Azadirachta indica and Antigonon leptopus leaves extract was determined using 96-well microtitre plate by the microbroth dilution method. [13] Dilutions were made in the range of 3000 µg/ml to 23.43 µg/ml and from 80 µg/ml to 0.078 µg/ml, respectively, for Azadirachta indica and Antigonon leptopus leaves extract, respectively, in Sabouraud dextrose broth previously standardized with fungal inoculum. DMSO served as the control, and Fluconazole was used as a standard. The Sabouraud dextrose broth, along with for Azadirachta indica and Antigonon leptopus leaves

extract, with two-fold serial dilutions, were added to a 96-well microtiter plate. The plate was then placed in a BOD incubator (REMI Cl 16, Mumbai, India) and incubated at a temperature of 37°C. Turbidity was observed for a period of 7 days. The minimum inhibitory concentration was reported as the lowest concentration of extract that did not exhibit any noticeable growth after 7 days of the incubation.

III. RESULTS

The antifungal bioassays demonstrated varying degrees of growth inhibition among the tested strain of Candida albicans when exposed individually and in combinations with each other. Table 1 denotes significant differences in fungal inhibition based on the concentration of Azadirachta indica and Antigonon leptopus leaves extract. Fig. 1 and Fig. 2. Shows the clear zone of inhibition for individual effects of Azadirachta indica and Antigonon leptopus leaves extract against Candida albicans. Fig. 3 shows the effects of different combinations of Azadirachta indica leaves extract with Antigonon leptopus against Candida albicans. Comparing the results from each well, as indicated in Table 2, it was observed that the maximum inhibition was achieved when Azadirachta indica leaves extract was combined with Antigonon leptopus leaves extract. It was observed that, when low doses of each extract were combined with each other, a notable zone of inhibition was seen as compare to the zone of inhibition observed for individual extracts. Table 3 denotes that Candida albicans growth was inhibited at the minimal inhibitory concentrations of 500 µg/ml for both Azadirachta indica leaves extract and Antigonon leptopus leaves extract, respectively.

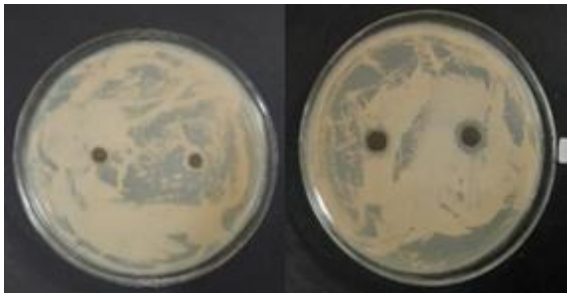

Fig.1

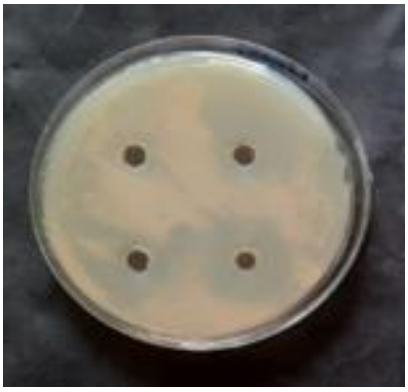

Fig.2

Fig.3

Sr No	Drug	Conc (µg/ml)	Zone of Inhibition(mm)			Mean (±SD)	P-Value
1	AIE	200	-	-	-	-	<0.05
		300	-	-	-	-	
		400	8.5	8.8	8.2	8.5±0.244[a]	
		500	12.5	12.0	11.9	12.13±0.262[a]	
2	ALE	350	-	-	-	-	<0.05
		400	11	11.3	11.5	11.2±0.205[b]	
		450	12.2	12.3	12.0	12.1 ±0.124[b]	
		500	14.3	14.0	14.5	14.2±0.205[b]	
3	STD	50	9.8	10	10.2	10±0.2	N/A
4	CTRL	50	0.0	0.0	0.0	0.0±0.0	N/A

Table 1. Determination of Antifungal Activity of Azadirachta indica and Antigonon leptopus leaves ethanolic extract

SN	Combination	AIE µg/ml	ALE µg/ml	ZOI (mm)			Mean ZOI±SD	P-value
1	Combination 1	200	200	11.9	11.1	11.2	11.4±0.35[c]	<0.05
2	Combination 2	300	300	12.3	12.9	12.6	12.6±0.24[d]	
3	Combination 3	500	500	23.5	23.1	23.2	23.27±0.21[e]	
4	STD(µg/ml)	25	N/A	13.8	14.1	14.0	13.97±0.15	N/A
5	CTRL (µl)	50	N/A	0.0	0.0	0.0	0.0 ± 0.0	N/A

Table 2. Determination of Antifungal Activity of different combinations of Azadirachta indica and Antigonon leptopus leaves extract

SN	AIE (µg/ml)	Remark	ALE (µg/ml)	Remark
1	2000	---	4000	---
2	1000	---	2000	---
3	500	---	1000	---
4	250	+++	500	---
5	125	+++	250	+++
6	62.5	+++	125	+++
7	31.25	+++	62.5	+++
8	15.625	+++	31.25	+++
9	STD	---	STD	---
10	CTRL	+++	CTRL	+++

Table 3 Determination of Minimum inhibitory concentration of Azadirachta indica and Antigonon leptopus leaves extract

IV. DISCUSSION

Several studies have revealed neem efficacy as a powerful antifungal agent. Herbal remedies have been used for the treatment of skin diseases since ancient times[13]. Despite the developmental growth in the field of medicine, the use of herbs in aromatherapy has remained rather popular[14]. The purpose of the study is to measure and analyse the effectiveness of neem leaf ethanolic extract combined with Antigonon leptopus leaves extract against Candida albicans, responsible for candidiasis. Several investigations have recognised the importance of Azadirachta indica and Antigonon leptopus plant leaves and their constituents as antifungal agents. The present study has revealed that Minimum inhibitory concentration effective in preventing the growth of the selected fungal species was 500 µg/mL for both Azadirachta indica and Antigonon leptopus leaves extract. However, it can be difficult to compare our MIC results to those of previous publications, since leaves extract composition can vary greatly depending on factors such as plant chemotype, endophytic microbial presence, geographical location, plant age, and process of extract preparation. Although the minimum inhibitory concentrations (MICs) of selected leaves extracts are higher than those of the conventional drug Fluconazole, it is important to consider the significant adverse effects of synthetic medicines such as azoles. However, the combination of Azadirachta indica and Antigonon leptopus might be a source of new antibiotic compounds and has a significant scope for antimicrobial research. The results of the present research support further analysis of Antigonon leptopus for purification in order to isolate the specific active constituent and to perform toxicology studies and in-vivo studies to identify its utilization in herbal drug formulations.

CONCLUSION

Ethnobotanical research has recently increased again as people look for new phytochemicals that may have medical uses. Multiple studies have recognized the importance of Azadirachta indica and Antigonon leptopus leaves extract and their constituents as antimicrobial agents, while no study investigated the possible synergistic effects in combination. The selected leaves extracts, when combined, exhibited

significant inhibitory effects on the investigated Candida-related diseases. The spread of candida albicans related disease species is mutually decelerated by the mixture of Azadirachta indica leaves extract and Antigonon leptopus leaves extract. The leaves extract of Azadirachta indica and Antigonon leptopus had exceptional fungicidal efficacy in all but was shown at considerably elevated concentrations. Therefore, it can be concluded that the antifungal effects of both extracts against Candida albicans were linked with their chemical conformation. Specifically, the terpenoids found in the leaves of Azadirachta indica and Antigonon leptopus, the study suggests the possible occurrence of a synergistic phenomenon that might be due to multimodal action.

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