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Original Research Article

In Vitro Evaluation of the Anticandidal Activity of Selected Medicinal Plant Extracts

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ABSTRACT

Candidiasis is one of the most prevalent and important opportunistic fungal infections caused by *Candida* species like *Candida albicans*, *C. glabrata*, and *C. krusei*. Suspected Candida infections are usually treated with amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and echinocandins. Potential risk of emergence of azole resistant strains of *Candida* isolates warrants careful selection of an antifungal drug for therapy. Medicinal plants possess activity against infectious agents like bacteria, virus and fungi. The objective of this study is to evaluate the *in vitro* activity of common Indian medicinal plants like *Aloe vera*, neem and tulsi leaves against *Candida* sp. isolates obtained from clinical samples. The clinical samples collected include nail scrapings, skin scrapings, vaginal swabs and oral swab. In the laboratory, the samples were cultured and *Candida* sp. were isolated and identified. Antifungal Disk Diffusion Susceptibility Testing was performed. Aqueous and alcoholic extracts of the medicinal plants were prepared and Minimum Inhibitory Concentration was determined against *Candida* sp. The overall number of drug resistant *Candida* isolates was found to be 35 out of 180 isolates, which gives the overall resistant strains as 19 % and all the resistant isolates were of *C. tropicalis* only. In conclusion, it can be shown that alcoholic extracts of *Aloe vera* leaves have more anticandidal activity followed by the aqueous extract. Alcoholic extract of neem leaves also have shown similar effect on *Candida sp*.

Key Words: *Candida sp.*, antifungal drugs, Disk Diffusion Susceptibility Testing, medicinal plants, broth microdilution, Minimum Inhibitory Concentration.

INTRODUCTION

Candidiasis is one of the most prevalent and important opportunistic fungal infections caused by *Candida* species like *Candida albicans*, *C. glabrata*, and *C. krusei*. Different forms of candidiasis affect various parts of the body, such as the oral and genital mucosa, skin, bronchus, gastrointestinal tract, and lungs. (Marzieh *et al*., 2016). [1] The frequent use of broad-spectrum antibiotics, central venous catheters, urinary catheters, prosthetic devices and increased frequency of abdominal surgery in intensive care unit (ICU) patients, put patients at a high risk of infection with *Candida* species. (Ravinder *et al*., 2016). [2]

Suspected Candida infections are usually treated with amphotericin B, flucytosine, fluconazole, (Claudio *et al*., 1991) [3] itraconazole, (Canadian Itraconazole study group, 1996) [4] voriconazole and echinocandins. (Peter *et al*., 2016). [5] However, a study by Shyamala and Prashanth (2014) [6] highlight the potential risk of emergence of azole resistant strains of *Candida* isolates. This study warrant careful selection of an antifungal drug for therapy.

Medicinal plants possess activity against infectious agents like bacteria, virus and fungi. (Armando *et al*., 2013). [7] The objective of this study is to evaluate the *in vitro* activity of common Indian medicinal plants like *Aloe vera*, neem leaves and tulsi against *Candida* sp. isolates obtained from clinical samples.

MATERIALS AND METHODS

The present study was conducted during a period of one year from September 2016 to September 2017 at School of Health Sciences, Kannur University. The clinical samples were obtained from the patients having the symptoms of candidiasis, attending a Tertiary care Hospital in North Kerala.

The clinical samples collected include nail scrapings, skin scrapings, vaginal swabs and oral swab and were isolated and identified based on standard methods. [8]

Antifungal Disk Diffusion Susceptibility Testing

Antifungal Disk Diffusion Susceptibility Testing was performed using Mueller-Hinton Agar (Himedia M173) + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium – with pH between 7.2 and 7.4. Antimicrobial Disks for fluconazole (FLC 25 mcg/disc), voriconazole (VRC 1 mcg/disc), itraconazole (IT 10 mcg/disc) and amphoterecin B (AP 100 units/disc) were used. The plates were inverted and placed in an incubator set to 35°C. Each plate was examined after 24 hours of incubation. [9]

Extraction technique of medicinal plants

Authentication of plant material was done from Arya Vaidyasala, Kottakkal before performing extraction. The leaves of *Aloe vera*, *Azadirachta indica* and *Ocimum sanctum* were used.

The plant materials were dried in the hot air oven for 72 hours and powdered for homogenisation using mixer grinder. The extraction was done in Soxhlet apparatus using water and ethanol as solvents. It was filtered through fine nylon cloth in order to remove debris and kept in bottles. (Stephen, 2015) [10]

A pilot study was done to test the activity of the plant extracts on *C.albicans* and *C.tropicalis* using the agar well diffusion method on RPMI 1640 (Himedia M1972). Interpretation of the test was done by observing the presence or absence of zone of inhibition around the wells. A zone of inhibition with a diameter of 12 mm or above was considered as sensitive and a zone of inhibition with a diameter less than 12 mm was considered as resistant. (Das *et al.*, 2010). [11]

Broth microdilution test procedure

Aqueous and alcoholic extract of 10 % concentration was used as the stock solution. The broth micro dilution test was performed by using sterile, disposable 96 well microdilution plates. 50 µl SD (Sabourauds Dextrose) broth was added to 8 wells. 50 µl of the plant extract was added to the first well. Mixed well and transferred 50µl of the suspension to the second well and so on till the seventh well. From the seventh well, 50 µl of the suspension was discarded. The eighth well is kept as control without any plant extract. Each well is inoculated with 50 µl of the inoculums suspension. The first well contains the highest concentration of the plant extract ie. 5 mg in 100 µl and the seventh well contains the lowest concentration of the plant extract ie. 0.0781 mg in 100 µl. Now, the control well contains only 50 µl of the SD broth and 50µl of the inoculums suspension. The microdilution plates were incubated at 37°c for 48 hours and observed for growth. The growth in each well is compared with that of the control well. In each series of wells, the last tube which shows clear supernatant was considered to be without any growth and taken as MIC (Minimum Inhibitory Concentration) value.

RESULTS

During the study period, a total of 325 clinical samples were collected including nail scrapings, skin scrapings, vaginal swabs and oral swab. From these

samples, 180 *Candida* isolates were obtained. A total of 84 *C.albicans* isolates were obtained and all the isolates were shown to be sensitive to voriconazole, fluconazole, itraconazole and amphoterecin B by antifungal disk susceptibility test. A total of 92 *C.tropicals* were obtained and out of these, 35 isolates were resistant to voriconazole, fluconazole and amphoterecin B and 57 isolates were sensitive to these drugs. All the *C.tropicalis* isolates were sensitive to itraconazole. All the 4 *C.glabrata* isolates were shown to be sensitive to voriconazole, fluconazole, itraconazole and amphoterecin B.

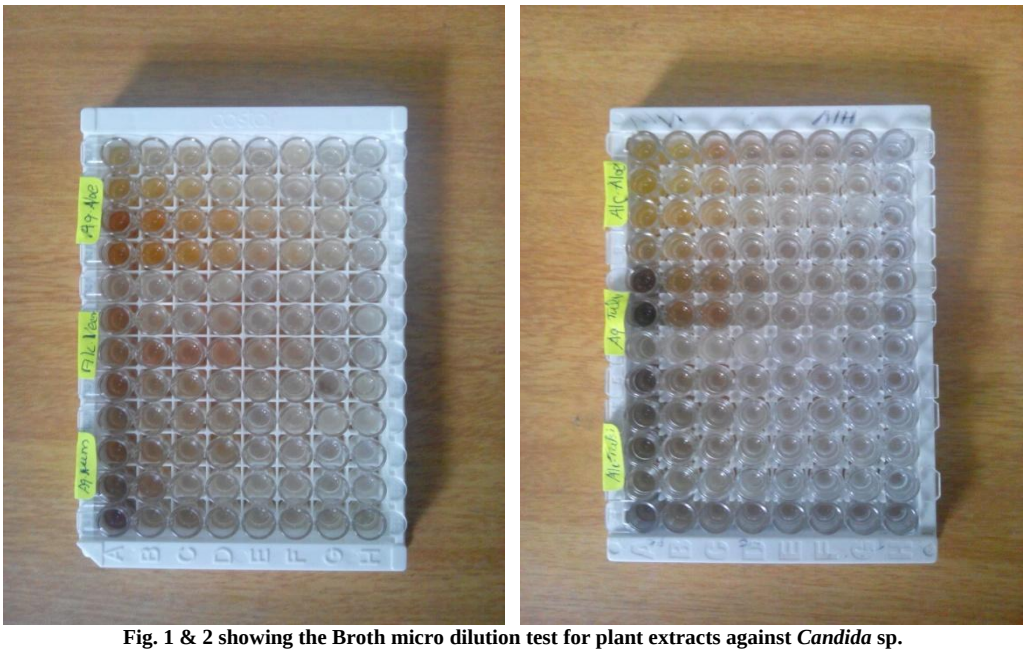

Fig. 1 & 2 showing the Broth micro dilution test for plant extracts against *Candida* sp.

Statistical analysis was done to find out the average MIC shown by the plant extracts against different species of *Candida* along with standard deviation.

Table 1. Statistical analysis for the MIC of plant extracts

Species	Plant extract	Method of extraction	N	Mean MIC	Std. Deviation
C.albicans	*Aloe*	aqueous	84	.245536	.1420210
		alcoholic	84	.264137	.1626625
	Neem	aqueous	84	.718006	.3422570
		alcoholic	84	.329241	.2029553
	Tulsi	aqueous	84	.747768	.3598138
		alcoholic	84	1.741071	.7345383
C.tropicalis	*Aloe*	aqueous	92	.232677	.0785345
		alcoholic	92	.224185	.0778820
	Neem	aqueous	92	1.929348	.6260443
		alcoholic	92	.266644	.0820218
	Tulsi	aqueous	92	.230978	.0784788
		alcoholic	92	.363451	.2211028
C.glabrata	*Aloe*	aqueous	4	.234375	.090211
		alcoholic	4	.351563	.19661
	Neem	aqueous	4	.625	.441942
		alcoholic	4	.429688	.234375
	Tulsi	aqueous	4	.9375	.360844
		alcoholic	4	.9375	1.052032

DISCUSSION

It is important to note that in this study, the overall number of drug resistant *Candida* isolates was found to be 35 out of 180 isolates, which gives the overall resistant strains as 19 % and all the resistant isolates were of *C.tropicalis* only. Comparison of antifungal susceptibility of

different *Candida* spp. by Yu-Shin *et al*., (2006) [12] has found that *C. glabrata* is more resistant to fluconazole than other *Candida* spp., but in the present study, *C.tropicalis* was found to be more resistant.

The mean MIC of aqueous and alcoholic extract of *Aloe vera* on *Candida albicans* was found as 0.24 and 0.26 respectively. The analysis did not show any significant difference between the study by Mbajiuka *et al*., (2014) [13] as MIC of both aqueous and alcoholic extract of *Aloe vera* on *Candida albicans* was 0.50. The mean MIC of aqueous and alcoholic extract of *Aloe vera* on *Candida tropicalis* was 0.23 and 0.22 respectively. For *C.glabrata*, the mean MIC value for aqueous extract of Aloe vera was 0.23 and that of alcoholic extract was 0.35. None of these differences can be considered as significant. This work has proved that *Aloe vera* leaf extract has good anticandidal activity.

The mean MIC of aqueous Neem extract on *C.albicans* was 0.72 and that of alcoholic neem extract was 0.33. *C.albicans* showed sensitivity to neem aqueous extracts in concentrations of 0.05-0.15 and to neem alcoholic extracts in concentrations of 0.025-0.0375. (Nayak *et al*., 2011) [14] For *C.tropicalis*, the values were 1.92 and 0.26 respectively. *C.glabrata* has given mean MIC values as 0.625 and 0.42. The present findings might help to find evidence of anticandidal action of neem leaf extracts.

The mean MIC of aqueous extract of Tulsi against *Candida albicans* was 0.74 and that of alcoholic extract was 1.74. The literature (Prof. (Dr) Mithra *et a*l., 2012) [15] also supports the result obtained. The mean MIC value for aqueous extract of Tulsi on *C.tropicalis* was 0.23 and for alcoholic extract, it was 0.36. Against *C.glabrata*, the mean MIC values were 0.93 for both the extracts. Tulsi extract also has anticandidal activity.

CONCLUSION

This study highlights the fact that there is a risk of emergence of azole resistant strains of Non albicans *Candida* (NAC) like *C. tropicalis*, which are increasingly being isolated from clinical specimens. In conclusion, it can be shown that alcoholic extracts of *Aloe vera* leaves have more anticandidal activity followed by the aqueous extract. Alcoholic extract of neem leaves also have shown similar effect on *Candida sp*.

REFERENCES

1. Marzieh Aghazadeh, Abed Zahedi Bialvaei, Mohammad Aghazadeh *et al*. Survey of the Antibiofilm and Antimicrobial Effects of *Zingiber officinale* (*in Vitro* Study). Jundishapur J Microbiol. 2016 February. 9(2): e30167.
2. Ravinder Kaur, Megh Singh Dhakad, Ritu Goyal, Rakesh Kumar. Emergence of non-albicans *Candida* species and antifungal resistance in intensive care unit patients. Asian Pac J Trop Biomed. 2016. 6(5): 455–460.
3. Claudio Viscoli, Elio Castagnola, Francesca Fioredda *et al*. Fluconazole in the Treatment of Candidiasis in Immunocompromised Children. Antimicrobial agents and Chemotherapy. Feb. 1991. Vol. 35, No. 2. p. 365-367.
4. Canadian Itraconazole study group, T W Austin, M Steben, M Powell *et al*. Short - course itraconazole in the treatment of *Candida* vulvovaginitis: A multicentre Canadian study. Can J Infect Dis. 1996; 7(2): 110-114.
5. Peter G. Pappas, Carol A. Kauffman, David R. Andes *et al.* Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clinical Infectious Diseases; 62(4): e1–50.
6. R. Shyamala and Prashanth K Parandekar. Identification and *In vitro* Azole resistance of *Candida* species isolated from Oropharyngeal Candidiasis in Human Immunodeficiency Virus infected patients. Int. J. Curr. Microbiol. App. Sci. (2014) 3(10) 816-822.
7. Armando Herrera-Arellano, Edgar O. López-Villegas, Aida V. Rodríguez - Tovar *et al.* Use of antifungal saponin

sc-2 of *Solanum chrysotrichum* for the treatment of vulvovaginal candidiasis: *in vitro* studies and clinical experiences. Afr J Tradit Complement Altern Med. (2013) 10(2):410-417.

8. Sachin C Deorukhkar and Santosh Saini. Laboratory approach for diagnosis of candidiasis through ages. Int. J. Curr. Microbiol. App. Sci. (2014) 3(1): 206-218.
9. Deorukhkar S.C, Saini S, Mathew S. Perspectives on antifungal susceptibility testing in *Candida.* Int J Infect Trop Dis. 2014; 1(2):51-59.
10. Stephen Olaribigbe Majekodunmi. Review of extraction of medicinal plants for pharmaceutical research. Merit Res. J. Med. Med. Sci. November, 2015.Vol. 3(11) pp. 521-527.
11. K. Das, R. K. S. Tiwari and D. K. Shrivastava. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research. 18 January, 2010. Vol. 4(2), pp. 104-111.
12. Yu-Shin Chiu, Shan-Chwen Chang, Po-Ren Hsueh *et al.* Survey of amphotericin B susceptibility of *Candida* clinical isolates determined by Etest. J Microbiol Immunol Infect. 2006; 39:335-341.
13. Mbajiuka Chinedu Stanley, Obeagu Emmanuel Ifeanyi, Okwandu Godson Eziokwu. Antimicrobial effects of *Aloe vera* on some human pathogens Int. J. Curr. Microbiol. App. Sci. (2014) 3(3): 1022-1028.
14. Nayak Aarati, Nayak Ranganath N, G Soumya B *et al.* Evaluation of Antibacterial and Anticandidal Efficacy of Aqueous and Alcoholic Extract of Neem (*Azadirachta indica*) an *in vitro* Study. International Journal of Research in Ayurveda & Pharmacy; IJRAP 2011, 2(1); pp 230-235.
15. Prof. (Dr) Mithra N Hegde, Dr. Shishir Shetty, Dr. Mahalaxmi Yelapure, Amit Patil. Evaluation of Antimicrobial Activity of Aqueous and Hydro-Alcoholic Curcuma Longa Extracts against Endodontic Pathogens. IOSR Journal of Pharmacy. Mar.-Apr. 2012, Vol. 2(2) pp: 192-198.

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