ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 01, pp.11825-11828, January, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EVALUATON OF ANTIFUNGAL PROPERTIES OF *ACORUS CALAMUS* (L)

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ARTICLE INFO

Article History:
Received 17th October, 2014
Received in revised form
25th November, 2014
Accepted 28th December, 2014
Published online 31st January, 2015

Key words:

Acorus calamus,
Antifungal drugs,
Dermatophytes,
Lyophilization.

ABSTRACT

Common Sweet Flag or *Acorus calamus* is a medicinal plant from the Acoraceae family. Tamil name is Vashambu. It is a tall perennial wetland monocot with scented leaves and rhizomes which have been used in medicinal field. *In vitro* studies indicate that even those plants / plant parts / extracts that have been already evaluated for their antimicrobial efficacy have to be re-evaluated adopting these more precise and advanced assay methods. Present study was Isolation and maintaining the standard ATCC strains and clinical fungal samples. Preparation of lyophilized seitz aqueous and ethanolic extract from *Acorus calamus* rhizome. Find the antifungal activity of rhizome extraction of *Acorus calamus* using micro dilution method (M 38-P) with the RPMI-1640 medium. Find the Minimum Inhibitory Concentration (MIC) of rhizome of *Acorus calamus* for standard strains and clinical isolations of *Candida albicans, Microsporum gypsum, Trichophyton rubrum* and *T. Mentagrophytes.* Compare the Minimum Fungicidal Concentration of rhizome of *Acorus calamus* with few standard antifungal drugs such as Amphotericin B, Itraconazole and Fluconazole. The results of the present study was lyophilized ethanolic extract of Acorcus calamus rhizome also exhibited fungicidal on both standard and clinical strains showing MIC value of 12.5 µg/ml for ATCC and 1.56 µg/ml for clinical. The efficacy of the lyophilized extract can be considered highly remarkable as the MIC value of standard medicines Amphotericin B, Itraconazole and Fluconazole on *Candida albicans and Trichophyton mentegrophytes and Trichophyton rubrum of* Clinical strains and as well as ATCC strains.

INTRODUCTION

Acorus calamus or Common Sweet Flag is a plant from the Acoraceae family. Tamil name is Vashambu. It is cultivated in many countries to satisfy the demand for its essential oil which is used for flavoring and in the perfumery and pharmaceutical industries (**Abanmi, 2008**). The purpose of the present study was to evaluate the in vitro antifungal susceptibilities of clinical isolates and standard cultures of *Candida albicans, Microsporum gypsum, Trichophyton rubrum* and *T. mentagrophytes* with standard strains by broth microdilution techniques as per National Committee for Clinical Laboratory standards guidelines and to compare the efficacy of Seitz filtered and lyophilized (aqueous and ethanolic) extracts of rhizome of *Acorus calamus* with Amphotercin B, Fluconazole and Itraconazole as standard antifungal agent. (**Bodey, 1992**) *In vitro* studies indicate that even those plants / plant parts / extracts that have been already evaluated for their antimicrobial efficacy have to be re-evaluated adopting these more precise and advanced assay methods.

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MATERIALS AND METHODS

In vitro Antifungal Assay

In vitro cultivation of test fungi

The following standard ATCC and Clinical cultures were maintaininge *Candida albicans (*ATCC 90029), *Microsporum gypseum* (ATCC 24102), *Trichophyton mentagrophytes* (ATCC 9533) and *Trichophyton rubrum* (ATCC 28188).

Preparation of lyophilized seitz filtered aqueous extract from *Acorus calamus* rhizome

30g of the powdered rhizome were ground in 150 ml of double distilled water and the mixture was stored in 10°C. The mixture was filtered through sterile gauze to get a whole aqueous (**Rajarajan, 2003**). The extract obtained was centrifuged at 3000 rpm for 10 minutes. The supernatant was seitz filtered by using a seitz filter of pore size 0.2µm. The sterile extract was then transferred into a lyophilization flask and kept sterility ensured for freezing at –80°C in deep freezer. The frozen sample was then loaded onto a lyophilizer to

remove the water content. The powder obtained as a result of lyophilization was transferred to sterile 5ml vials and stored at -20°C until further use.

Preparation of lyophilized seitz filtered ethanolic extract from *Acorus calamus* rhizome

The method of preparation of seitz filtered lyophilized ethanolic extract of *Acorus calamus* rhizome was similar to the method followed in aqueous extract. The difference was that instead of water, 70% ethanol was used to prepare the extract.

Serial two-fold dilution of drugs

Serial two-fold dilutions were prepared according to NCCL's approved documents (M38-P). Eight test tubes were taken and first tube was filled with 2ml of sterile RPMI-1640 medium and remaining seven tubes were filled with 1ml of medium and weighed (200µg) (**Espinel, 2001**). Whole lyophilized extract was dissolved in the first tube and mixed well and serially diluted. The resultant dilutions of the extract was 12.5µg, 6.25µg, 3.125µg, 1.56µg, 0.78µg, 0.39µg 0.165µg and 0.165µg per ml. Same diluting procedure was carried out for standard antifungal agents (Amphotericin B, Itraconazole and Fluconazole) and isolated pure chemicals (A, B, C and D) were weighed (100µg) and dissolved in DMSO and serially diluted.

***In vitro* antifungal assay by broth Micro dilution method (M 38-P)**

The *in vitro* antifungal assay of Seitz filtered and lyophilized whole extracts (aqueous and ethanolic) from Indian Medicinal plant, *Acorus calamus* was performed by broth micro dilution as per NCCLS (M 38-P) guidelines.

Inoculum preparation

Inoculum suspension of *Candida albicans* was prepared by picking subcultures on SDA plates and it is dissolved in 5ml of sterile 0.85% NaCl. It was diluted into 1:50 using RPMI-1640 medium of sterile saline into seven-day-old culture tube. (Pfalle, 2004). This suspension was vigorously shaken and vortexed for 15 minutes. Then it was transferred aseptically into sterile tubes. The spore density was adjusted spectrophotometrically at 530nm. Finally it was diluted into 1:50 using RPMI-1640 medium. The prepared inoculum were quantified by plating on SDA.

Test procedure

Three controls were maintained - control 1) Positive control dry free medium with fungal inoculums; control 2) Blank which consists of dry free medium only without fungal inoculums and control 3) Medium with DMSO and fungal inoculums. Each microtitre well containing sterile RPMI-1640 medium, (**Espinel, 1996**) add serially two-fold diluted drugs (standard and test) to the respective well. Then add standard fungal inoculums was delivered to the entire well except blank control. All the microtitre plates were incubated at 24°C for Dermatophytes and 37°C for *Candida albicans* and examined after 21 to 26, 46 to 50 and 70 to 74 hours of incubation.

RESULTS

Characteristics of seitz filtered aqueous extracts and lyophilized aqueous extracts of *Acorus calamus* rhizome

The physical characteristics of seitz filtered aqueous extract done as per the methodology outlined in the Methodology chapter earlier from *Acorus calamus* rhizome are Bitter taste, Yellowish brown, Transparency, Non – viscous and pH 6.5. The physical characteristics of lyophilized aqueous extract of the seitz filtered done as per the methodology outlined in the Methodology chapter earlier from *Acorus calamus* rhizome are Brown in coulour, Fine crystal and Sticky.

Characteristics of seitz filtered ethanolic extracts and lyophilized ethanolic extracts of *Acorus calamus* rhizome

The physical characteristics of seitz filtered ethanolic extract done as per the methodology outlined in the Methodology chapter earlier from *Acorus calamus* rhizome are Bitter taste, Yellowish brown, Transparent, Non – viscous and pH 6.9. The physical characteristics of lyophilized ethanolic extract of the seitz filtered done as per the methodology outlined in the Methodology chapter earlier from *Acorus calamus* rhizome are Brown, Fine powder and Sticky.

In vitro antifungal activity for *Candida albicans* (Table 1)

The lyophilized aqueous extract fungicidal property had been observed with an MIC / MFC value for *Candida albicans* at 1.56 µg/ml for ATCC strain and 1.56 µg/ml for clinical strain. The lyophilized ethanolic extract of *Acorus calamus* rhizome also exhibited fungicidal on both standard and clinical strains showing MIC value of 1.56 µg/ml for ATCC and 1.56 µg/ml for clinical.

Table 1. Comparative Table for *Candida albicans*

Drug	Candida albicans (ATCC 90029) MIC µg/ml	Candida albicans (Clinical) MIC µg/ml
Amphotercin B	+	+
	3.12	3.12
Itraconazole	-	-
	0.00	0.00
Fluconazole	-	-
	0.00	0.00
Aqueous lyophilized extract	+	+
	1.56	1.56
Ethanolic lyophilized extract	+	+
	1.56	1.56

The efficacy of the lyophilized extract can be considered remarkable as the MIC value of Amphotericin B (pure chemical) on *Candida albicans* ATCC strain was only 3.12 µg/ml and while the MIC / MFC value for clinical strain was one dilution lower than the lyophilized extracts of 3.12 µg/ml.

***In vitro* antifungal activity for Dermatophytes**

It has been found that lyophilized aqueous extract fungicidal activity on all the three dermatophytes both ATCC and clinical at a concentration range of 12.5 – 1.56 µg / ml (Table 2,3 & 4) and ethanolic extract fungicidal activity is restricted to *Microsporum gypseum* (Table 2).

Table 2. Comparative Table for *Microsporum gypseum*

Drug	Microsporum gypseum (ATCC 24104) MIC µg/ml	Microsporum gypseum (Clinical) MIC µg/ml
Itraconazole	+	+
	6.25	6.25
Fluconazole	+	+
	6.25	6.25
Aqueous lyophilized extract	+	+
	12.5	12.5
Ethanolic lyophilized extract	-	-
	12.5	12.5

Table 3. Comparative Table for *Trichophyton mentegrophytes*

Drug	Trichophyton mentagrophytes (ATCC 9533) MIC µg/ml	Trichophyton mentagrophytes (Clinical) MIC µg/ml
Itraconazole	+	+
	12.5	6.25
Fluconazole	+	+
	12.5	6.25
Aqueous lyophilized extract	+	+
	3.12	1.56
Ethanolic lyophilized extract	+	+
	3.12	12.5

Table 4. Comparative Table for *Trichophyton rubrum*

Drug	Trichophyton rubrum (ATCC 28188) MIC µg/ml	Trichophyton rubrum (Clinical) MIC µg/ml
Itraconazole	+	+
	6.25	6.25
Fluconazole	+	+
	6.25	6.25
Aqueous lyophilized extract	+	+
	6.25	1.56
Ethanolic lyophilized extract	+	+
	6.25	1.56

The *in vitro* antifungal assay, the efficacy of the lyophilized aqueous and ethanolic extracts are equal to that of the standard anti dermatophytic drugs formed in the experiments namely of Itraconzole and Fluconazole, i.e., 12.5 µg / ml – 6.25 µg/ml (Table 2,3 & 4).

The present study of the lyophilized ethanolic extract of Acorcus calamus rhizome also exhibited fungicidal on both standard and clinical strains showing MIC value of 12.5 µg/ml for ATCC and 1.56 µg/ml for clinical. The efficacy of the lyophilized extract can be considered highly remarkable as the MIC value of standard medicines Amphotericin B, Itraconazole and Fluconazole on *Candida albicans and Trichophyton mentegrophytes and Trichophyton rubrum of* ATCC strain and as well as clinical strains (Table 3 & 4).

Conclusion

The climatic conditions of India favours fungal infections especially of the *Candidiases* and *Superficial mycoses.* The effect of the lyophilized extracts were highly encouraging. The *in vitro* efficacy of these extracts namely aqueous and ethanolic were very impressive as these extracts exhibited fungicidal activity on *Candida albicans*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* (standard/clinical). The MIC range was between 12.5 µg/ml for ATCC and 1.56 µg/ml for clinical. The efficacy of the lyophilized extract can be considered "highly remarkable" compared to the standard medicines Amphotericin B, Itraconazole and Fluconazole on *Candida albicans,* *Trichophyton mentegrophytes and Trichophyton rubrum* in ATCC strains as well as Clinical strains.

REFERENCES

Abanmi. "Characteristics of superficial fungal infections in the Riyadh region of Saudi Arabia. 2008. *International Journal of Dermatology*, "Vol.47(3): pp. 229-235.

Aghighi. S. "Antibacterial and antifungal survey in plants used indigenous herbal- medicine of southeast region of Iran." 2004. *Journal of Biological Science*, Vol.4 No.3: pp.405-412

Bodey, G. P. 1992. Azole antifungal agents. *Clin. Infect. Dis.*, 14(Suppl 1):S161-S169.

Chaurasia, S.C. and Jain, P.C. 1978. "Antibacterial activity of essential oils of four medicinal plants". *Indian J. Hosp. Pharm.,* 15(6):166–168.

Degreef, H., Heeres, J., Borgers, phd M. 2006. Antifungal Azoles for skin disorders expert opinion on therapeutic patents, volume 16, number 9, September 2006, pp. 1235-1253(19).

Espinel – Ingroff A., Fothergill A., Peter J., Rinaldi, M. G. and Walsh, T. J. 2001. "Testing conditions for determination of Minimum fungicidal concentrations of new and established antifungal agents for *Aspergillus spp*" NCCLS collaborative study. *J. Clin. Microbiol*., 2001; 40: 3204 – 3208.

Espinel – Ingroff A., Pfaller M., Erwin M. E. and Jones, R. N. 1996. Interlaboratory evaluation of E-test method for testing antifungal susceptibilities of pathogenic yeasts to

five antifungal agents by using casitone agar and solidified RPMI 1640 medium with 2% glucose. *Journal of Clinical Microbiology*, 848 – 852.

Kannan. P., C. Janaki and G.S. Selvi, 2006. "Prevalence Of Dermatophytes And Other Fungal Agents Isolated From Clinical Samples" *Indian Journal of Medical Microbiology, 24 (3):212-5.*

Kapoor L.D. 2001. "Traditional uses of medicinal plants. Ayurvedic Medicinal plants", 2001, vol 6, pp. 327

Pfalle M A, Messer S A, Boyken L, Hollis R J, Rice C, Tendolkar S. and Diekema D J. *In vitro* activities of voriconazole, posaconazole, and fluconazole against 4, 169 clinical isolates of *Candid spp*. And *Cryptococcus neoformans* collected during 2001 & 2002 in the APTEMIS global antifungal surveillance program. Diagnostic Microbiology and infectious disease 2004; 48: 201 – 205.

Rajarajan S, Shanbhag Gayathri N, Anand D, Thyagarajan S. P. and Subramanian S. A study on the *in vitro* Antifungal properties in the aqueous extracts of unripe and ripe fruits of *Terminalia catappa linn*. *Indian Journal of Applied Microbiology,* 2003; 3 (1): 67 – 69.

Romano, C., Ghilardi, A. and Fimiani, 2006, "Dystrophic onychomycoses due to *Microsporum Gypseum* mycoses", volume 49, number 4, July 2006, pp. 335-337(3).

Souwalak Phongpaichit, Nongyao Pujenjob, Vatcharin Rukachaisirikul and Metta "Antimicrobial activities of the crude methanol extract of *Acorus calamus* Linn". Songklanakarin *J. Sci. Technol.,* 2005, 27(Suppl. 2) : 517-523

Venkatesan G., A. J. A. Ranjit Singh, A. G. Murugesan, C. Janaki and S. Gokul shankar, "*Trichophyton rubrum* – the predominant etiological agent in human dermatophytoses in chennai, india". *African Journal of Microbiology Research* pp.

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