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IN VITRO ANTICANDIDAL ACTIVITY OF *PIMENTA DIOICA* (ALLSPICE) ESSENTIAL OIL AGAINST CLINICAL ISOLATES OF *CANDIDA ALBICANS* AND NON-ALBICANS *CANDIDA*.

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ABSTRACT

The antimicrobial property of volatile aromatic oils from medicinal as well as other edible plants has been recognized since antiquity. *Candida* species are an important cause of opportunistic infections in the oral cavity of immunocompromised patients and vaginal candidiasis. The antifungal activity of the essential oil of *Pimento dioica* (allspice oil) was investigated against 75 clinical isolates of *Candida albicans* and non-albicans *Candida*. Sensitivity profile of clinical isolates to undiluted and diluted (3:1, 2:2 and 1:3) allspice oil was evaluated by disc diffusion method. Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were evaluated by broth microdilution and broth macrodilution method. Allspice oil effectively inhibited all clinical isolates of *C. albicans* and non-albicans *Candida* with growth inhibition zones ranging from 24 to 44 mm. Allspice oil inhibited *C. albicans* growth with mean minimum inhibitory concentration (MIC) of 0.98 µl/ml (v/v) and 1.14 µl/ml (v/v) by broth micro dilution and broth macrodilution method, respectively. The clinical isolates of *C. albicans* required as high as 1.25 µl/ml (v/v) concentration of allspice oil for its inhibition by both methods. The isolates of non-albicans *Candida* showed MIC range of 0.15 – 2.50 µl/ml (v/v) by broth microdilution and 0.31 – 2.50 µl/ml (v/v) broth macrodilution. The isolates of *C. krusei* exhibited higher sensitivity to allspice oil than other isolates with lowest MIC of 0.15 µl/ml (v/v).

Key words: Antiyeast activity, medicinal plant, essential oil, *Pimento dioica*, pathogenic yeast

1. INTRODUCTION

In recent years, a significant worldwide increase in fungal infections has been reported in the medical literature. Fungi cause both superficial and internal mycoses. *Candida* spp. is an important cause of bloodstream infections and opportunistic infections in the oral cavity of immuno-compromised patients (Pfaller MA and Diekema DJ, 2002). *Candida* spp. is also the most common cause of vaginal candidiasis or thrush and approximately 80-90% of thrush cases are caused by *Candida albicans* with other species (Hammer KA et al. 1998). *Candida albicans* has accounted for virtually all mucosal candidiasis and responsible for about 60% of both superficial and systemic mycoses (White TC et al. 1998). However, in recent years this picture is complicated by the emergence of non-albicans *Candida* (NAC) species such as *Candida glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* which cause serious oropharyngeal candidiasis and occasionally esophageal candidiasis (Wingard JR,1995; Vazquez JA et al. 2000).

There are a limited number of antifungals available, most just providing fungistatic but not fungicidal effects. Continuous exposure to the

antibiotics and synthetic chemical drugs results in the development of resistance in the organisms. Fluconazole, itraconazole, voriconazole are the primary drugs used for treatment of serious fungal infections. However, decreased susceptibility among yeast and molds to these prime antifungal drugs have been noted and prompted a search for new drugs that may be effective in the management of patients with mycoses due to a wide range of filamentous fungi and yeast pathogens (Espinel-Ingroff A, 1998; Imhof A, 2004; Pina-Vaz C et al. 2005). The increasing ineffectiveness of these drugs and unavailability of alternative antimicrobials in developing countries causing spread of major infectious diseases.

Several compounds have been isolated from plants, which have antimicrobial or medicinal properties (Cowan MM, 1999). Essential oils derived from plants are well known in traditional medicine and proved to have insecticidal (Werdin Gonzalez JO et al. 2011), bactericidal (Patil SD and Kamble VA, 2011), fungicidal (Kamble VA and Patil SD, 2008; Zabka M et al, 2009) and nematicidal (Leela NK and Ramana KV 2000) effects. In addition, essential oils have also been used in various cancer treatments (Sylvestre M, 2006), food preservation (Patil SD and Kamble VA, 2011), aromatherapy (Wolfgang S and Michaela S, 2008), pharmaceutical (Maruyama N et al. 2005), fragrance (Isman MB et al. 2011) and cosmetic industries (Kejlova K et al. 2010).

Allspice oil is extracted from *Pimenta dioica* (L.) Merr (also known as *Pimenta officinalis,* family *Myrtaceae)*, by steam distillation of the crushed and dried berries. The evergreen dried fruits and leaves of *Pimenta dioica* tree are used world wide as valuable spices. They are commonly known as allspice, Jamaica pepper and Pimento. This spice possesses the characteristic flavour and aroma of clove, nutmeg, cinnamon and black pepper, all combined in this one spice, hence named allspice. The therapeutic properties of the allspice oil are analgesic, antimicrobial, antioxidant, antiseptic, carminative, muscle relaxant, stimulant, tonic and in menopause. Allspice oil can be helpful for the indigestion, flatulence, rheumatism and nausea. Further the essential oil can help in cases of depression, nervous exhaustion, tension, neuralgia and stress (Agarwal, 1997; Harikumar B et al. 2010). Eugenol has been reported to be the major and inhibitory component of allspice oil. Allspice oil also contains cineole, levophellandrene, caryophyllene, and humulene (Karapinar, M and Aktug SE 1987; Zabka M et al. 2009; Padmakumari KP et al. 2011). Allspice oil possesses very high radical scavenging activities, hence can be utilized as a natural antioxidant for health benefits (Feng X et al. 2010; Padmakumari KP et al. 2011). Currently, very little information is available on its comparative antifungal activity on the growth and physiolology of human pathogenic yeasts either *in vitro* or *in vivo*. There needs a high-quality studies investigating the use of allspice for any indication.

The objective of the present study was to evaluate the *in vitro* inhibitory activity of allspice oil against clinical isolates of *Candida albicans* and non-albicans *Candida*. This study shows for the first time that *in vitro* anticandidal activity of allspice oil determined by using broth microdilution and macrodilution methods for clinical isolates of *Candida*.

2. MATERIALS AND METHODS

2.1 Essential Oils

The allspice oil used in the present investigation was kindly provided by Synthite Industrial Chemicals Limited, Kolenchery (Kerala, India). The sterility of the oils was checked by inoculating a loopful of oil on potato dextrose agar and nutrient agar slants, and then assessing the growth. The essential oil was stored in the dark at 25^{o}C when not in use. Different concentrations of allspice oil with DMSO (25%, 50% and 75%) were prepared for experiments.

2.2 Organisms

75 clinical isolates of the pathogenic yeasts including *Candida albicans* (n = 28), *C. krusei* (n = 7), *C. glabrata* (n = 9), *C. parapsilosis* (n = 7), *C. tropicalis* (n = 12), *C. pseudotropicalis* (n = 6), *C. guilliermondii* (n = 3) and *C. stellatoidea* (n = 3) were isolated from various clinical specimens such as oral swab, vaginal discharge and blood. Each isolate was originated from a different patient with clinical manifestations. Clinical isolates were

identified to the species level based on morphological criteria, carbohydrate assimilation profile, and germ tube test in serum and chlamydospores production on corm meal agar (Koneman EW and Roberts GD, 1985). Isolates were maintained on Sabouraud dextrose agar (Hi-Media, Mumbai) at 4^{o}C in refrigerator until used in the study. Prior to testing, each isolate was checked for purity and viability.

2.3 Inoculum Preparation

To prepare inoculum, a small amount of growth was taken from 24 h old culture of the respective organism grown on SDA slant and inoculated into 5 ml of 0.85% sterile saline. The resulting inoculum density was adjusted to 0.5 McFarland standard to yield a suspension of 1×10^6 to 5×10^6 cells/ml (NCCLS, 2002).

2.4 Anticandidal Sensitivity Testing By Disc Diffusion Method

Disc diffusion assay of allspice oil was performed as described by Bauer et al., (Bauer AW et al. 1966). In short 90 mm-diameter plates containing Sabouraud dextrose agar (Hi-Media, Mumbai) at a depth of 4 mm were used. The agar surface was seeded with inoculum by using a sterile swab. Sterile disc (SD 067, Hi-Media, Mumbai) of 6 mm diameter was impregnated with 20 µl of undiluted oil (100%) and diluted (25%, 50%, 75% in DMSO) was placed at center of seeded agar surface. The plates were then left undisturbed for 30 min to allow diffusion of the essential oil into the agar and incubated at 35 ^{0}C for 24 h. The inhibition zone was measured in millimeter and the assay was carried out three times for each isolate tested. Isolates with zone size ≥ 28 mm were classified as strongly sensitive, with a zone diameter of < 28 to 16 mm as moderately sensitive, with a zone diameter of < 16 to 12 mm as weakly sensitive and isolates with zone diameter of < 12 mm as resistant (Elgayyar et al. 2001).

2.5 MIC & MFC Studies

Minimum inhibitory concentrations (MIC) and Minimum fungicidal concentrations (MFC) of allspice oil were determined by broth microdilution and broth macrodilution method (NCCLS, 2002) with some modifications.

2.5.1 Broth Microdilution Method

Stock solution of allspice oil (10 µl/ml) was prepared in sterile Sabouraud dextrose broth (Hi-Media, Mumbai). 0.15% (w/v) bacteriological agar (Hi-Media, Mumbai) was added as a stabilizer (Mann CM and Markham JL 1998) of the oil water mixture. Serial two fold dilutions of stock solution of essential oil was prepared over the range of 0.02 – 10 µl/ml (v/v) with a final oil concentration range 0.01 – 5.0 µl/ml (v/v) in 96-well microtitre plates. A freshly grown yeast suspension in Sabouraud dextrose broth was standardized to 1 x10^6 cells/ml (0.5 McFarland standard). A working yeast inoculum suspension of 1x10^4 cells/ml was prepared by diluting the stock inoculum (1 x 10^6) 1:100 with sterile Sabouraud dextrose broth. Sabouraud dextrose broth containing 0.15% agar without essential oil served as growth control. 100 µl yeast suspension was added to each well. Well containing only the Sabouraud dextrose broth with 0.15% agar without microorganism was used as sterility control. The lowest concentration of oil that inhibited visible growth after incubation at 35^{o}C for 48 ± 2 h without shaking was taken as minimum inhibitory concentration (MIC). Minimum concentration of oil that inhibited 50%, 70% and 90% of the isolates tested were defined as MIC_{50}, MIC_{70} and MIC_{90}, respectively. To determine minimum fungicidal concentration (MFC), a loopful of broth was removed from each well and spot inoculated onto SDA plate and after incubation at 35^{o}C for 48 h, the lowest concentration of oil that inhibited the complete growth was considered as MFC. The concentration of allspice oil fungicidal for 90% of the clinical isolates tested was defined as MFC_{90}.

2.5.2 Broth Macrodilution Method

A range of doubling dilutions of allspice oil from 0.02 – 10 µl/ml (v/v) with a final oil concentration range 0.01 – 5 µl/ml (v/v) was prepared in Sabouraud dextrose broth in round bottom sterile glass tubes (12 × 75 mm). Bacteriological agar was included at a concentration of 0.15% (w/v) to enhance oil solubility. A working inoculum suspension of 1×10^4 cells/ml was added to each

tube except sterility control. Sabouraud dextrose broth containing 0.15% agar without essential oil served as growth control. The tubes were then incubated at 35^0C for 48 ± 2 h without agitation and observed for the presence or absence of visible growth. The MIC was defined as the lowest concentration of oil inhibiting visible growth.

3. RESULTS

3.1 Anticandidal Activity by Disc Diffusion Method:

In the present study, we have examined the anticandidal properties of allspice oil *in vitro* by disc diffusion method. The mean inhibition zones (MIZ) and inhibition zone range obtained against eight different *Candida* species is shown in Table 1. All the clinical isolates of *Candida* were strongly inhibited by undiluted allspice oil with 22 – 44 mm inhibition zone range. Oil of allspice was highly potent against the isolates of *C. krusei* (MIZ = 37 mm) and was comparatively least potent to the isolates of *C. parapsilosis* (MIZ = 30 mm). Isolates of *C. albicans, C. tropicalis,* and *C. pseudotropicalis* were inhibited with mean inhibition zone of 32 mm, while the isolates of *C. glabrata* and *C. guilliermondii* exhibited a 26 – 39 mm and 30 – 32 mm range, respectively with mean inhibition zone of 31 mm. All the clinical isolates were sensitive even to the 3:1, 2:2 and 1:3 diluted allspice oil.

Table 1.0: ***Inhibition zones obtained by disc diffusion method of the allspice oil assayed against eight different Candida species.***

Species (No. of isolates)	Zone of inhibition (mm)							
	4:0 (100% oil)		3:1 (75% oil)		2:2 (50% oil)		1:3 (25% oil)	
	MIZ	IZR	MIZ	IZR	MIZ	IZR	MIZ	IZR
C. albicans (n = 28)	**32**	**29 – 38**	**28**	**25 – 32**	**25**	**22 – 28**	**20**	**16 – 25**
C. glabrata (n = 9)	**31**	**26 – 36**	**26**	**21 – 31**	**22**	**18 – 26**	**18**	**13 – 23**
C. tropicalis (n = 12)	**32**	**26 – 34**	**27**	**22 – 31**	**23**	**19 – 27**	**19**	**16 – 24**
C. parapsilosis (n = 7)	**30**	**24 – 34**	**26**	**21 - 29**	**22**	**17 – 26**	**19**	**15 – 22**
C. pseudotropicalis (n = 6)	**32**	**30 – 34**	**27**	**26 – 29**	**23**	**22 – 25**	**20**	**18 – 23**
C. krusei (n = 7)	**41**	**38 – 44**	**37**	**33 – 40**	**33**	**29 – 37**	**27**	**24 – 30**
C. guilliermondii (n = 3)	**31**	**30 – 32**	**27**	**27 – 28**	**23**	**23 – 24**	**17**	**17 – 18**
C. stellatoidea (n = 3)	**35**	**34 – 36**	**27**	**27 – 28**	**23**	**23 – 23**	**19**	**18 – 20**
Total (n = 75)	**32**	**24 – 44**	**28**	**21 – 40**	**24**	**17 – 37**	**20**	**13 – 30**

MIZ, Mean Inhibition Zone; IZR, Inhibition Zone Range; 4:0 represents undiluted oil; 3:1 represents 3 parts oil and 1 part DMSO solvent; 2:2 represents 2 parts oil and 2 parts DMSO solvent; 1:3 represents 1 part oil and 3 parts DMSO solvent

3.2 Evaluation of Anticandidal Activity by Broth Microdilution Method:

In our study, various concentrations of allspice have been used to determine the MIC_{90} and MFC_{90} by broth microdilution assay (Table 2). *C. krusei* was found to be the most susceptible among the *Candida* species, requiring lowest amount of allspice oil for its inhibition, with its MIC_{90} of 0.62 µl/ml. The least susceptible of the *Candida* species to allspice oil was the *C. parapsilosis and C. tropicalis*, with MIC_{90} of 2.50 µl/ml. MIC range for the isolates of *C. albicans* was found to be 0.31-1.25 µl/ml allspice oil with MIC_{90s} at 1.25 µl/ml. Much of the isolates of *C. albicans* showed good consistency with respect to MIC requirement, with exception of few isolates that showed 2 to 4 fold variations in their MIC values. However, allspice oil showed optimum inhibitory activity against *C. glabrata, C. pseudotropicalis*, *C. guilliermondii* and *C. stellatoidea* at 1.25 µl/ml concentration.

Table 2.0: ***Minimum inhibitory concentrations (MIC) obtained by broth microdilution of the allspice oil assayed against eight different Candida species.***

Species (No. of isolates)	MIC (µl/ml)				
	MIC Range	MIC_{50}	MIC_{70}	MIC_{90}	Mean MIC
C. albicans (n = 28)	**0.31 – 1.25**	**1.25**	**1.25**	**1.25**	**0.98**
C. glabrata (n = 9)	**0.62 – 1.25**	**0.62**	**1.25**	**1.25**	**0.93**
C. tropicalis (n = 12)	**0.31 – 2.50**	**0.62**	**1.25**	**2.50**	**1.20**
C. parapsilosis (n = 7)	**0.62 – 2.50**	**0.62**	**1.25**	**2.50**	**1.12**
C. pseudotropicalis (n = 6)	**0.62 – 1.25**	**1.25**	**1.25**	**1.25**	**1.09**
C. krusei (n = 7)	**0.15 – 0.62**	**0.15**	**0.15**	**0.62**	**0.30**
C. guilliermondii (n = 3)	**1.25 – 1.25**	**1.25**	**1.25**	**1.25**	**1.25**
C. stellatoidea (n = 3)	**1.25 – 1.25**	**1.25**	**1.25**	**1.25**	**1.25**
Total (n = 75)	**0.15 – 2.50**	**1.25**	**1.25**	**2.50**	**1.01**

MIC = minimum inhibitory concentration expressed in µl /ml).

3.2 Evaluation of Anticandidal Activity by Broth Macrodilution Method:

Table 3 illustrated the MIC and MFC *in vitro* susceptibility of anticandidal activity by allspice oil assessed by broth macrodilution at different concentrations. The broth macrodilution study also showed that the *C. krusei* was most susceptible to allspice oil at minimum concentration of 0.62 µl /ml, whereas *C. parapsilosis* was least susceptible to allspice oil. In the result, a wide MIC range was found in *C. parapsilosis* and *C. tropicalis* by broth macrodilution assay between 0.62 – 2.50 µl /ml. In contrast, the present results revealed the most susceptible species *C. krusei* showed narrow MIC range between 0.15 – 0.62 µl /ml by both broth macrodilution assay. For the majority of *Candida* species tested MIC_{90s} were 1.25 µl/ml and oil was effective in inhibiting growth of different *Candida* species at concentrations from 0.15 – 2.50 µl/ml. Mean MIC_S for all species of *Candida* were found to be greater than 1.0 µl/ml, with the exception of *C. krusei* for which mean MIC was found to be much lower (mean MIC 0.36 µl/ml) showing its higher sensitivity towards allspice oil.

Table 3.0: ***Minimum inhibitory concentrations (MIC) obtained by broth macrodilution of the allspice oil assayed against eight different Candida species.***

Species (No. of isolates)	MIC (µl/ml)				
	MIC Range	MIC_{50}	MIC_{70}	MIC_{90}	Mean MIC
C. albicans (n = 28)	**0.31– 1.25**	**1.25**	**1.25**	**1.25**	**1.14**
C. glabrata (n = 9)	**0.62 – 1.25**	**1.25**	**1.25**	**1.25**	**1.04**
C. tropicalis (n = 12)	**0.62 – 2.50**	**1.25**	**1.25**	**1.25**	**1.24**
C. parapsilosis (n = 7)	**0.62 – 2.50**	**1.25**	**1.25**	**2.50**	**1.37**
C. pseudotropicalis (n = 6)	**0.62 – 1.25**	**1.25**	**1.25**	**1.25**	**1.09**
C. krusei (n = 7)	**0.15 – 0.62**	**0.31**	**0.31**	**0.62**	**0.36**
C. guilliermondii (n = 3)	**1.25 – 1.25**	**1.25**	**1.25**	**1.25**	**1.25**
C. stellatoidea (n = 3)	**0.62 – 1.25**	**0.62**	**1.25**	**1.25**	**0.93**
Total (n =75)	**0.15 – 2.50**	**1.25**	**1.25**	**2.50**	**1.05**

MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration (MIC and MFC values are expressed in µl/ml).

4. DISCUSSION

C. albicans is a harmless commensal dimorphic yeast-like fungus in healthy humans, which can cause superficial as well as life threatening systemic infections under immunocompromised states (Koneman EW and Roberts GD, 1985). *C. albicans* can infect virtually all body sites because of its high adaptability to different host niches by the activation of appropriate sets of genes in response to complex environmental signals (Vijaya M et al. 2001). In this context, our aim was to evaluate the possible therapeutic potential of allspice oil against this human dimorphic commensal organism, which can become a facultative pathogen under altered physiological situations. In the light of this fact and with a intension to manage the *C. albicans*, our previous investigation (Kamble VA and Patil SD, 2008) assessed the antifungal activity of several essential oils and reported the strong antifungal activity of allspice oil against reference strains of *C. albicans* (MTCC-227, MTCC-3017 & NCIM-3100), *C. glabrata* (MTCC-3019), *C. krusei* (MTCC-231), *C. blanki* (MTCC-624), *C. cylindracea* (MTCC-1908), *C. tropicalis* (MTCC-184) with inhibition zones ranging from 24 to 46 mm. In the present investigations therapeutic potential of allspice oil is assessed directly against the *Candida* spp. isolated from different patient with clinical manifestations. The results were extremely promising and showed good consistency with strong inhibitory potential towards the clinical isolates of *C. albicans, C. glabrata, C. krusei* and *C. tropicalis* with 32 mm, 31 mm, 41 mm and 32 mm mean inhibition zones, respectively.

Previous studies to assess the antifungal effects of *Pimento dioica* essential oil against pathogenic and toxinogenic fungi which are the major economic problems of crop and food production, have demonstrated the superior antifungal effect *P. dioica* oil against *Fusarium oxysporum*, *Fusarium verticillioides*, *Penicillium expansum*, *Penicillium brevicompactum*, *Aspergillus flavus* and *Aspergillus fumigates* (Zabka M et al., 2009). Essential oil of *P. dioica* was found to inhibit the growth of *C. albicans* isolated from spoiled meat products with 34.5 mm inhibition zone and 0.2 % v/v as MIC (Gochev VK and Girova TD, 2009). *In vivo* studies of an aqueous suspension of *P. dioica* in mice revealed its ability to reduce yeast-induced hyperpyrexia and also showed antiulcer and cytoprotective activity (Adnan J et al. 2002). Earlier, strong antimicrobial effects of *P. dioica* essential oil and its pure aromatic volatile eugenol against the yeast *C. albicans* were reported (Hoferl M et al, 2009).

The outstanding feature of allspice oil was the effective inhibition of all the clinical isolates of *Candida* species isolated from blood, vaginal and oral thrush with 24 – 44 mm inhibition zones and 0.15 – 2.50 µl/ml MIC range. This superior antiyeast effect could be due to high abundance of eugenol and methyl-eugenol in allspice oil. The effective doses of allspice oil against the isolates of *C. albicans* and non-albicans *Candida* were in the range of 0.31 – 1.25 µl/ml and 0.15 – 2.50 µl/ml, respectively and could be comparable to MIC values of the most frequently used synthetic fungicides for a treatment serious human mycoses (Espinel-Ingroff A, 1998). Thus, allspice oil could represent a new generation of anti-Candida preparations. The essential oil of allspice demonstrates great potential as a novel antifungal compound with potent *in-vitro* fungicidal activity against *C. albicans*, an opportunistic pathogen responsible for both superficial and systemic mycoses. The results of this *in vitro* assay are extremely hopeful and the additional *in vivo* clinical studies are required to assess therapeutic efficacies of the compounds of allspice oil for treating mucosal candidisis and *Candidal* infections in immunocompromised patients.

MFC values for allspice oil were similar to MIC results. MFC_{90} range for different *Candida* species was found to be 0.62 – 2.50 µl/ml. MIC values reported in this study could be much significant for the inhibition of germ tube formation, an important virulence factor in the pathogenesis of *C. albicans* (Pina-Vaz C, 2005). Over the past two decades, an increasing trend in the number of vaginal infections attributable to yeasts other than *C. albicans* has emerged. Of these non-albicans species, *C. tropicalis* and *C. glabrata* appear to be the most important. The change in incidence pattern of yeast vaginitis can be expected to impact greatly on the treatment of this condition.

Many currently used drug therapies (e.g., imidazoles) for *C. albicans* vaginitis do not adequately eradicate non-albicans species (Horowitz BJ et al. 1992). In the present investigation *C. albicans* and non-albicans species such as *C. tropicalis* and *C. glabrata* were inhibited at 1.25 µl/ml concentration of allspice oil. In results, oil could be promising in designing the remedies for the treatment of vaginal candidiasis.

Candida krusei is an emerging fungal nosocomial pathogen primarily found in the immunocompromised and those with hematological malignancies and recipients of blood and marrow transplants. *Candida krusei* has been recognized as a potentially multidrug- resistant (MDR) fungal pathogen. Its intrinsic resistance to fluconazole and decreasing susceptibility to other anti-fungal agents such as flucytosine and amphotericin B are problematic (Pfaller MA et al. 2008). Most promising and surprising fact in this study was the high susceptibility of *Candida krusei* to allspice oil extracted from *P. dioica*, at a concentration of 0.62 µl /ml.

Although essential oils are irritant and allergenic, they are prescribed in low dilutions for topical treatments on skin and even on mucous (Chaumont JP, 2003). As allspice oil in this investigation showed strong anticandidal behaviour at a low concentration, the oil may be used for the treatment of cutaneous candidiasis. When topically applied, it could be a valid substitute to synthetic drugs. In the present study, essential oil was insoluble in the test medium. However, addition of 0.15 % bacteriological agar before sterilization was found to give better solubility of allspice oils into the test medium with no turbidity. 0.15 % agar has been previously used as an emulsifier by Mann and Markham (Mann CM and Markham JL, 1998) for susceptibility testing of bacteria. Furthermore, there was found to be a good agreement between the results obtained by broth microdilution and broth macrodilution method. In comparison to broth macrodilution, MIC values shown by broth microdilution were either same or 2 fold less. MIC_{90s} for clinical isolates assessed by both methods shows more sensitivity of *C. krusei* isolates, less sensitivity of *C. parapsilosis* isolates and moderate sensitivity of *C. albicans, C. glabrata, C. pseudotropicalis, C. guilliermondii* and *C. stellatoidea* isolates to allspice essential oil.

5. CONCLUSION

In conclusion, allspice oil shows significant promise as a potential therapeutic agent for the treatment of superficial and mucosal candidiasis including vaginal candidiasis. *In vitro* results indicate anticandidal efficacy of allspice oil at low concentration. But certain clinical trials are needed to determine the usefulness of allspice oil *in vivo*.

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