



Faculty of Agriculture & Veterinary Medicine, Thamar University

Yemeni Journal of Agriculture & Veterinary Sciences

Available online at; WWW.YJAVS.COM

DOI: xxxx.xxxx

***In vitro* evaluation of antifungal activity of some medicinal plants extracts against vaginal candidiasis (*Candida albicans*)**

Najla M. Baghza

Department of Microbiology, Faculty of Medicine and Health Sciences, Dhamar University, Dhamar, Yemen, Corresponding author e-mail; baghza2006@yahoo.com

ABSTRACT

Article history:

[Received](#)

31 March 2014

[Accepted](#)

25 April 2014

Vaginal candidiasis is the most frequent infection of the female genital tract and is caused by the overgrowth of *Candida albicans*. This study was carried out to determine the prevalence of *Candida albicans* among vaginal candidiasis cases in Dhamar City, Yemen and to evaluation (*in vitro*) the effect of some different medicinal plants extracts : *Ocimum basilicum* , *Camellia sinensis*, *Lawsonia inermis* and *ziziphus spina* for their inhibitory effects against *Candida albicans*. Study was performed on 80 women attending Al-Amoma hospital, General Dhamar hospital and some of private clinics in Dhamar City, Yemen. All specimens were diagnosed clinically as vaginal candidiasis. The vaginal swab protocol was designed to collect the vaginal specimens and the isolates were identified according to a standard procedure and cultured on Sabouraud dextrose agar. Ethanol and aqueous solvents were used for prepare the plants extracts and measured their activity by disc diffusion method. The results showed that *C. albicans* was susceptible to the different extracts, of which aqueous and ethanol extract in variation of inhibitory effects.

Keywords: Vaginal candidiasis, Plants extracts, *Candida albicans*, Dhamar, Yemen.

INTRODUCTION

The incidence of fungal infections has increased significantly in the last 20 years (Poeta *et al.*, 1999). *Candida albicans* is the most frequently isolated invasive fungal pathogen in humans, with the majority of

infections being localized to the urogenital or oropharyngeal tracts of the patient (Fidel and Sobel , 1996). It is the major medically important species and etiologic agent of many fungal infections occurring in oral or vaginal cavity (Stenderup,1990 ; Costa *et al.*, 2006). *C. albicans* has evolved into a

commensal organism as well as an opportunistic pathogen, implying that it is routinely present in what is considered to be a healthy mucosal microflora (Sobel, 1998).

Vaginal Candidiasis or Vulvovaginal candidiasis, is caused by the overgrowth of *Candida albicans*, in the vaginal flora (Sobel *et al.* 1998). The vaginal pH is typically 4 to 4.5, which distinguishes candidiasis from trichomoniasis or bacterial vaginosis (where the pH is elevated) (Sobel, 2007). Vulvovaginal candidiasis is classified by the World Health Organization (WHO) as a pathological condition that is frequently sexually transmitted (STD) and it has been considered to be an important worldwide public health problem (Gunther *et al.*, 2014).

Medicinal plants are renewable in nature unlike the synthetic drugs that are obtained from non-renewable sources of basic raw materials such as fossil sources and petrochemicals (Samanta *et al.*, 2000). Due to all these advantages, plants continue to be a major source of new lead compounds. Nowadays, the indiscriminate use of commercial antimicrobial drugs has caused multiple drug resistance in human pathogenic microorganisms (Aliero *et al.*, 2008). This situation forced scientists to search for new and effective antimicrobial agents to replace the current regimens. With an increase in the antibiotic-resistant strains of fungi, traditional plants are being investigated for their antimicrobial and medicinal values. Traditional uses of plants have led to investigating their bioactive compounds, which have resulted in the

detection of a significant number of therapeutic properties (Jacquelyn, 2002).

The purpose of this study was to determine the prevalence of *Candida albicans* among vaginal candidiasis cases in Dhamar City, Yemen. And evaluation of antifungal activity of some medicinal plants extracts against *Candida albicans*.

MATERIALS AND METHOD

Isolation of *Candida albicans*

Study was performed on 80 women attending Al-Amoma hospital, General Dhamar hospital and some of private clinics in Dhamar City, Yemen. All specimens were diagnosed clinically as vaginal candidiasis. The collected of specimens was in the period of January to October (2013).

The Vaginal Swab Protocol was designed to collect vaginal specimens and the isolates were identified according to a standard procedure and cultured on Sabouraud dextrose agar (SDA, Oxoid) plates at 37 °C for 24-48 hrs to ensure optimal growth before testing. Complimentary tests such as germ tube test, corn meal agar media, and sugar fermentation test were carried out to differentiate between the *Candida albicans* from other *Candida* species (Mahon and Manuselis, 1995; Saigal *et al.*, 2011).

Preparation of the plants extracts

Four of medicinal plants were collected from different local retail markets of herbs in Dhamar City. Leaves of *Ocimum basilicum*, *Camellia sinensis*, *Lawsonia inermis* and *Ziziphus spina* were cleaned twice using distilled water. They were cut into pieces and grinded using a sterile electric grinding into fine powder form.

Aqueous extract

The fine-powdered of plants (200 gm) were infused in distilled water until complete exhaustion. The extract was then filtered using Whatman No. 1 filter paper, and the filtrate was then evaporated and dried using either a rotary evaporator at 60 °C. The concentrated plant material was then soaked in 10 ml of distilled water. The final material was stored in sterile bottles and kept in a freezer at -20 °C (Kandi *et al.*, 1994).

Ethanol extract

The fine-powdered of plants (200 g) were infused in 95% ethanol. The extracts were then filtered through Whatman filter paper No.1. The filtrate was evaporated and concentrated using a rotary vacuum evaporator. The concentrated plant material was then soaked in 10 ml 95% ethanol. Finally, kept at -20°C in sterile bottles (Fabry *et al.*, 1996; Tragoolpua, 1996).

Preparation of the extracts concentrations

The test samples from ethanol and aqueous plants extracts were prepared at different concentrations, 50mg/ml, 25mg of each plants. To prepare 100 mg / ml concentration, 1 g of the filtrate was reconstituted in 2 ml of each solvent (Ethanol and Distilled water). 50 mg/ml and 25 mg/ml concentration were prepared by adding equal volume of solvent and 100 mg/ml concentration earlier prepared, that

is, double fold dilution. Double fold dilution was carried out on the 50 mg/ml concentration to give 25 mg/ml (Adegoke *et al.*, 2013).

Inoculums preparation and disc diffusion method

Three to five colonies of *Candida albicans* was suspended in 2ml of sterile normal saline and vortexes. The sterile swab was dipped in suspension and swabbed on dried plates of Sabouraud's dextrose agar. 6 mm sterile filter paper discs were purchased and sterilized. These were placed and inoculated on dried Sabouraud's dextrose agar plates. 10µl of the extraction was placed on the disc. These plates were incubated at 37°C. Zone of inhibition was noted around the disc at 24 and 48 hrs. The positive control used in the study was 95 % ethanol (Supreetha *et al.*, 2011).

RESULTS

The present study was conducted to determine the prevalence of *Candida albicans* among vaginal Candidiasis cases in Dhamar City, Yemen. From eighty women cases that clinically diagnosed as vaginal candidiasis, *Candida albicans* was isolated in the eighty cases. Figure 1.

2

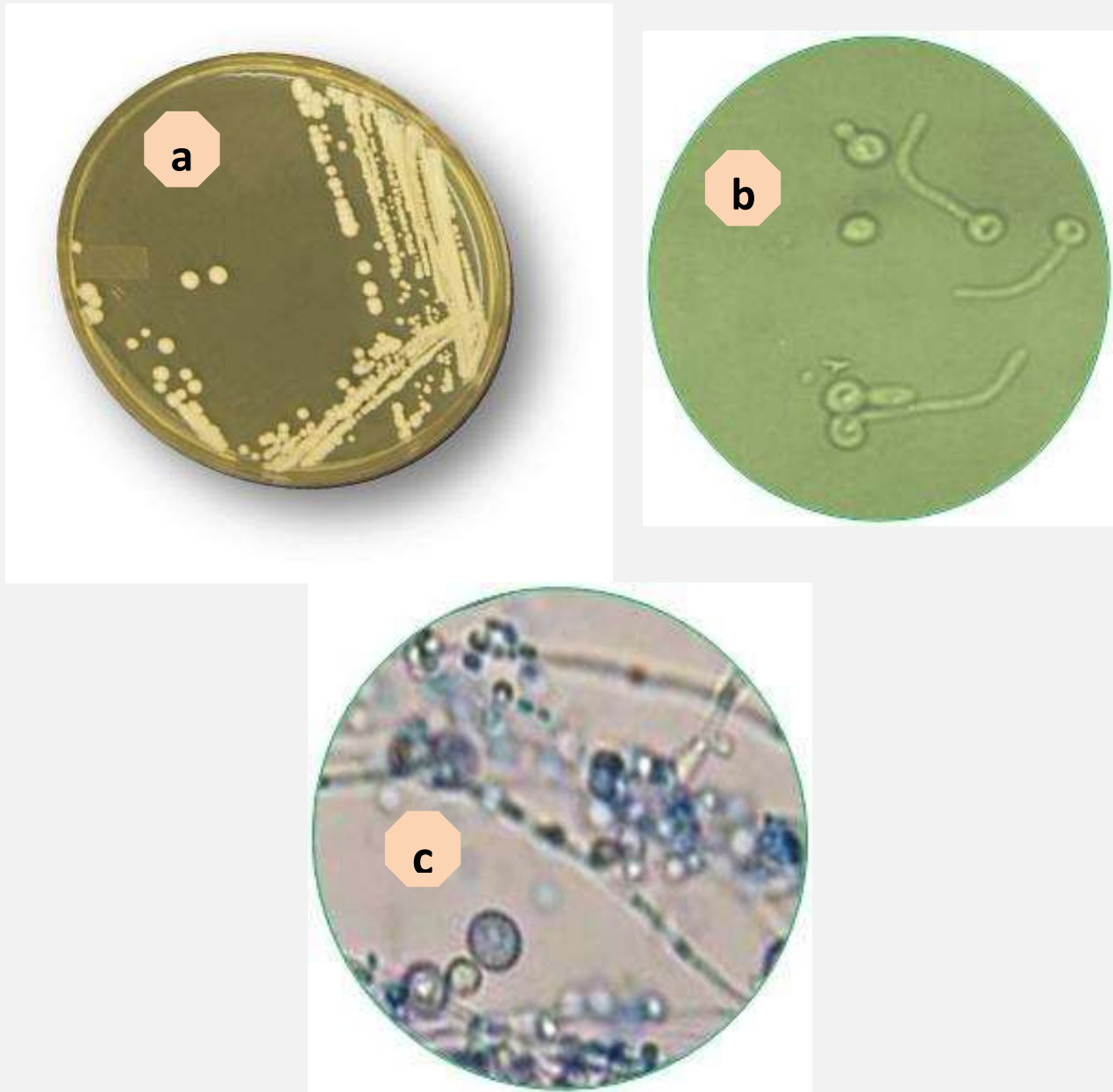


Figure. *Candida albicans* isolated from vaginal candidiasis
a: *Candida albicans* colonies on Sabouraud dextrose agar at 48h at 37C.
b: Germ tube formation in human serum $\times 40$.
c: Chlamyospore formation in corn meal broth media with Gram stain at 24 hours $\times 40$

Leaves of *Ocimum basilicum*,
Camellia sinensis, *Lawsonia inermis*
and *Ziziphus spina* were

used in this study for their evaluation
(*in vitro*) as antifungal activity against
Candida albicans, figure 2.



Figure 2- Medicinal plants which used in this study

- a: *Lawsonia inermis*
- b.: *Ziziphus spina*
- C: *Camellia sinensis*
- d: *Ocimum basilicum*

The results showed that *C. albicans* was susceptible to the four different plant extracts: *Ocimum basilicum*, *Camellia sinensis*, *Lawsonia inermis* and *ziziphus spina*, of which aqueous and ethanol extract, but it is clear that ethanol plants extracts showed more antifungal activity against *C. albicans* compared with aqueous extracts of the same plants. Among the four extracts

tested, The largest zones of inhibition were observed (ethanol extract) in *Lawsonia inermis* (34 mm at concentration 50%) followed by *Ocimum basilicum* (32 mm at the same concentration). While *Camellia sinensis* and *ziziphus spina* ethanol extracts where less effective compared to the other extracts (22 and 20 mm, respectively) as shown in Table 1.

Table 1: Antifungal activities of plant extracts against *Candida albicans*

Fungi	Zone of inhibition (mm)															
	<i>Ocimum basilicum</i>				<i>Camellia sinensis</i>				<i>Lawsonia inermis</i>				<i>Ziziphus spina</i>			
	A		E		A		E		A		E		A		E	
<i>C. albicans</i>	50% Con.	25% Con.	50% Con.	25% Con.	50% Con.	25% Con.	50% Con.	25% Con.	50% Con.	25% Con.	50% Con.	25% Con.	50% Con.	25% Con.	50% Con.	25% Con.
	25	13	32	18	17	9	22	13	27	14	34	18	16	11	20	15

A: Aqueous extract : E: Ethanol extract : Con. : Concentration of the extract.

DISCUSSION

Vaginal candidiasis is the most frequent infection of the female genital tract and is caused by the overgrowth of *Candida albicans*. This results showed that from eighty women cases that clinically diagnosed as vaginal candidiasis, *Candida albicans* isolated in the eighty cases. Sharanappa and Vidyasaga (2013) noted that: around 75% of adult women have at least one episode of vulvovaginal candidiasis (VVC) during their life, with prevalence of *C. albicans* in 70–90% . While Ringdahl (2000) noted that more than 50% of women over 25 age have at least one episode of

vaginal candidiasis. Pregnancy, contraception, and other causes of hormonal imbalance may affect the normal acidic environment of the vagina, increasing susceptibility to infection. Diabetes and diets rich in carbohydrates may also contribute to chronic yeast infections (de Leon *et al.*, 2002).

This result showed that *Ocimum basilicum*, *Camellia sinensis*, *Lawsonia inermis* and *ziziphus spina* were have antifungal activity properties against *Candida albicans*. Various studies confirm that : *Ocimum basilicum*, *Camellia sinensis*,

Lawsonia inermis and *Ziziphus spina* have antimicrobial effects (Chopra *et al.*, 1986 ; Abalaka *et al.*, 2010; Kulandhaivel & Palaniswamy, 2012 and Rahiman *et al.*, 2013) respectively. In recent years, multiple drug resistance in human pathogenic microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Davis, 1994 ; Martin & Ernst 2004). The World Health Organization (1993) estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80 % of the world's population. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena & Sharma 1999, Ahmad & Beg, 2001).

CONCLUSION

C. albicans was frequent infection of the vaginal candidiasis, in this study which was carried out to determine the prevalence of *Candida albicans* among vaginal candidiasis cases in Dhamar City. The present study renders medicinal plants like *Ocimum basilicum*, *Camellia sinensis*, *Lawsonia inermis* and *Ziziphus spina* to the field of dentistry which can be used as alternative medicines and adjunct to conventional therapy which will be a great help in developing countries with financial constraints and limited vaginal health care facilities for populations. This study was conducted *in vitro*.

ACKNOWLEDGMENTS

I would think all the staff member in hospitals in Dhamar City for their cooperation during the collection of the specimens.

REFERENCES

- Abalaka**, S. , Daniyan, S. and Mann, A. 2010. Evaluation of the antimicrobial activities of two *Ziziphus* species (*Ziziphus mauritiana* L. and *Ziziphus spinachristi* L.) on some microbial pathogens, *Afric. Jou. of Pharm.* 4(4): 135-139.
- Adegoke** , S. A., Agada, F. D. and Ogundipe, L. 2013. Antibacterial activity of methanol and ethanol leaf extracts of *Antidesma venosum* and *Lannea barteri*. *Africa. Jour. of Micro. Resear*, 7 (27) : 34-42.
- Ahmad**, I. and Beg, A.Z. 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J Ethno. pharmacol*, 74:113-123.
- Aliero**, A., Aliero, BL. and Buhari , U. 2008. Preliminary phytochemical and antibacterial screening of *Scadoxus multiflorus*. *Int J Pure Appl Sci*, 2 :13-15.
- Chopra**, RN., Nayar, SL. and Chopra, IC. 1986. *Glossary of Indian Medicinal Plants (Including the Supplement)*. Council of Scientific and Industrial Research. New Delhi.
- Costa**, C.R., Lemos, J.A , Passos, X.S., Araujo, C.R. Cohen, A.J., Souza, L.K. and Silva, M. R. 2006. Species distribution and

antifungal susceptibility profile of oral *Candida* isolates from HIV-infected patients in the antiretroviral therapy. *Mycopathologia*, 162:45–50.

Davis, J. .1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science*, 264: 375-382.

de Leon et al. 2002. Prevalence and risk factors for vaginal *Candida* colonization in women with type 1 and type 2 diabetes. *BMD Infectious Diseases*, 2: 1-4.

Fabry, W., Okemo, P. and Ansorg, R. 1996. Fungistatic and fungicidal activity of East African medicinal plants. *Mycoses*, 39:67-70.

Fidel, P.L. and Sobel, J.D. 1996. Immunopathogenesis of recurrent vulvovaginal Candidiasis. *Clin Micro Rev*, 9: (1): 335-348.

Gunther, L. , Martins, H. , Gimenes, F. , Abreu, A. and Consolaro, M. 2014. Prevalence of *Candida albicans* and non-albicans isolates from vaginal secretions: comparative evaluation of colonization, vaginal candidiasis and recurrent vaginal candidiasis in diabetic and non-diabetic women. *Sao Paulo Med J*, 132 (2):116-20.

Jacquelyn, GB. 2002. *Microbiology principles and exploration* . 5th ed. USA : John Wiley and Sons Inc.

Kandi, O. , Radwan, N.M, Hassan, A.B et al. 1994 : Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities. *J Ethnopharmacol.*, 44: 19-24.

Kulandhaivel, M. and Palaniswamy, M. 2012. *In vitro* antimicrobial activity of *Camellia sinensis* and *Myristica fragrans* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*. *Inter. Jour. of Pharma. & Biolo. Archi*, 3(3): 604-609.

Mahon, C.R. and Manuselis, G. 1995. *Textbook of diagnostic microbiology*. WB Saunders, USA.

Martin, W. and Ernst, E. 2004. Herbal medicines for treatment of fungal infections: a systematic review of controlled clinical trials. *Mycoses*, 47: 87-92.

Poeta, M.D., Bixel, A.S. and Barchiesi, F. 1999 : *In vitro* activity of dicationic aromatic 221 compounds and fluconazole against *Cryptococcus neoformans* and *Candida* spp. *J. Antimicrob. Chemother.*, 44:223-28.

Rahiman , F., Mahmad , N. , Taha , R. , Elias and Zaman , F. 2013. Antimicrobial properties of *Lawsonia inermis* syn. *Lawsonia alba* , *in vivo* and *in vitro*. *JFAE*, 11 (4):502-504.

Ringdahl, E. N. 2000. Treatment of recurrent vulvovaginitis candidiasis. *The American Academy of Family Physicians*. 61: 11.

Saigal, S. , Bhargava, A. , Mehra, K. and Dakwala, F. 2011. Identification of *Candida albicans* by using different culture media and its association potentially malignant and

malignant lesion. Contemp Clin Dent. 2 (3): 188–193.

Samanta, M.K. , Mukherjee, P.K. , Prasad, M.K. and Suresh, B. 2000. Development of natural products. Eastern Pharmacist. (2): 2-3.

Saxena, VK. and Sharma, RN. 1999. Antimicrobial activity of the essential oil of *Lantana aculeata*. Fitoterapia., 70: 67-70.

Sharanappa, R. and Vidyasaga G. 2013. Anti-Candida activity of medicinal plants. Jour. of Pharm & Pharmac. Scien, 5: 4- 9.

Sobel, J. D. 2007. Vulvovaginal candidosis. Lancet. 369 (9577): 1961-1971.

Sobel, J. D. , Faro, *et al.* 1998. Vulvovaginal candidiasis : epidemiologic, diagnostic, and therapeutic considerations. Americ.J. of Obstet. & Gyneco., 178 (2): 203-11

Sobel, J.D. 1998. Pathogenesis and Epidemiology of Vulvovaginal Candidiasis. Ann . Acad Sci., 544:547-557.

Stenderup, A. 1990. Oral mycology. Acta .Odontol. Scand., 48 (1):3–10.

Supreetha, S. , Sharadadevi , Mannur , Simon , S., Jain, J. , Tikare, SH. and Mahuli, M. 2011. Antifungal activity of ginger extract on *Candida albicans*: an *In-vitro* Study. Indi. J. 2 (2): 18-21.

Tragoolpua, K. 1996. Effect of the extract from eight species of medicinal plants on growth of selected plant pathogenic molds and dermatophytes. M.Sc. thesis. Department of Biology, Faculty of Sciences, Chiang Mai University, Chiang Mai, Thailand.

World Health Organization. 1993. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram. 28: 13Ð14.