Antifungal Activity of Some Plant Extract and Alum on yeast isolated from mouth lesions

Rasha Abd.AL-Mehdi Flayeh
University of Babylon ,Collage of Pharmacy

Abstract

In this study, 50 swabs were collected from patients suffering from oral thrush and mouth ulcers during the period from March /2009 to November /2009. *Candida albicans* formed the ma jor causative agents of these lesions followed by *Streptococcus mutance, Pseudomonas aeruginosa*,which do not studied in the research .

This study was conducted to investigate the sensitivity of these isolates toward two plants extracts (*Syzygium aromaticum* the common name is (Clove) , and *Peganum harmala*) and chemical material such as (Alum) as alone and in combination. Study also determined the MIC for each extract and alum. The study showed that Clove and *Peganum harmala* had high antimicrobial activity against all isolates even at low concentrations ; the MIC values are 0.25 mg/ml and 0.5 mg/ml respectively where as the MIC value of Alum is (10 mg/ml). Also the study showed synergism effect between these two extracts so the isolates were more sensitive toward *Peganum harmala* extracts when used with Alum but Clove become less activity when used with Alum in the same plate.

الخلاصة:

تضمنت هذه الدراسة 50 مسحة جمعت من اشخاص يعانون من بعض الاصابات في الفم القلاع و القرحة الفموية للفترة من اذار/2009 حتى تشرين الثاني/2009 . اظهرت الدراسة ان *Candida albicans* لها الدور الرئيسي في اصابة الفم .كما تم عزل بكتريا *Streptococcus mutance* وبكتريا *Pseudomonas aeruginosa* لكن لم يتم دراستها في هذا البحث .

لقد تم استخدام نوعين من المستخلصات النباتية (مستخلص ازهار القرنفل ومستخلص بذور الحرمل) ومادة كيمياوية مثل (الشب) لدراسة الفعالية المضادة لهذه المواد على *Candida albicans* ومدى حساسية هذه العزلات تجاه كل مادة لوحدها وتجاه خليط هذه المواد ، حيث اظهرت الدراسة ان كل المواد تمتلك فعالية مضادة عالية تجاه العزلات المستخدمة في هذه الدراسة حتى عند استخدامها بتراكيز واطئة حيث تراوح التركيز المثبط الادنى لمستخلص القرنفل (0.25) ملغم/مل ولمستخلص الحرمل (0.5) ملغم/مل اما لمادة الشب فهو 10 ملغم/مل . كما اوضحت الدراسة ان لخليط مادة الشب مع مستخلص الحرمل تاثير تازري حيث اظهرت جميع العزلات حساسية اكبر تجاه خليط هذه المواد عند استخدامها سوية في نفس الوسط الزرعي. كما اظهرت الدراسة أن هناك تاثير تضادي لخليط الشب مع مستخلص القرنفل فقد اظهرت العزلات مقاومة اكبر تجاه خليط هذه المواد عند استخدامها سوية في نفس الوسط الزرعي

Introduction

There are many lesions occur in the mouth like oral thrush and mouth ulcer. Bad mouth hygiene or a fungal infection (candidiasis or mouth thrush) cause white patches on the tongue and cheek commonly referred to as mouth ulcers that can be easily treated by anti-fungal medicines.(Hughes&Flynn,2004)

Oral thrush is a condition caused by the overgrowth of the *Candida albicans* fungus, commonly known as yeast, in the mouth. Candida is a parasitic type fungus that inhabits the intestines, genital tract, mouth, esophagus, and throat. Normally this fungus lives in healthy balance with the other bacteria and yeasts in the body, however, certain conditions can cause it to multiply, weaken the immune system further and cause an acute infection. Symptoms of oral thrush

ISSN 2072-3875

include creamy-looking white patches on the tongue and mucous membranes of the mouth. Oral thrush can affect both male and female, however it is seldom transmitted sexually. It is most common in babies and persons with compromised immune systems . Anyone who has been on long term antibiotic therapy or has taken antibiotics often probably has an overgrowth of Candida somewhere in the body. Antibiotics weaken the immune system and also destroy the "friendly" bacteria that normally keep Candida under control. Food allergies are often a contributing factor in cases of oral thrush. .(Balch&Balch , 2002)

Thrush is most commonly caused by the yeast *Candida albicans*. Less frequently, other forms of *Candida* can lead to thrush. These include *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis*, and *Candida glabrata*.(Papas, 2004)

This study was undertaken to detect the MIC values for some plant extracts (*Syzygium aromaticum* (Clove) and *Peganum harmala*) and chemical material (Alum) which can killed or reduce the *Candida albicans* isolates causing mouth ulcer or oral thrush. The antifungal activity of each substance alone and incombination was also studied. .

Materials & Method

Fifty cotton swabs were collected from the people who suffering from some lesions in mouth (oral thrush, mouth ulcer) from March /2009 to November /2009 , in screw capped tubes containing 10 ml Brain Heart Infusion broth, after that these samples were cultured on typical media (Nutrient agar contain cloromphenicol, Sabrouaud dextrose agar, Tobacco medium, rice meal, carbohydrate fermentation test, serum or plasma medium) .All samples incubated aerobically at 37C for 24-48hr then subjected to the diagnosis morphological and biochemical differential tests (Almuaamary,2007)

In this study two plants extracts (*Syzygium aromaticum* , flowers); *Peganum harmala* (seeds))and chemical material potassium alum were used . Plants extracted by alcohol to get an active compound and study its antifungal activity of all substances first as alone was used to (detect the MIC for each extract) and secondly studying the synergism effect between them, by using Muller- Hinton agar supplemented with different concentrations of these substances. Plates were supplemented with Clove, *Peganum harmala* and potassium alum each alone and by Clove and harmala mixture,Clove and Alum ,*Peganum harmala* and Alum mixture. (Sambrook, 2004)

All plates were inoculated with the yeast isolates using picking and patching method then incubated at 37C for 24 to 48 h.

Results & Discussion

This study was conducted to investigate the activity of two plants extracts (*Syzygium aromaticum* , and *Peganum harmala*) and chemical material (Alum) on growth of *Candida albicans* isolates from people suffering from some lesions (mouth ulcer or oral thrush). .

The results showed the antifungal activity of the two plants extracts (*Syzygium aromaticum* (Clove) and *Peganum harmala*) and potassium alum against these fungal isolates tables (1,2) . The results showed that all substances used had high antimicrobial activity even at low concentrations. The MIC value of Clove was 0.25mg/ml the MIC value of *Peganum harmala* was

ISSN 2072-3875

0.5mg/ml and MIC value of Alum was 10 mg/ml. These results agreed with that reported by (Fu,*et.al*,2007) who found that clove had significant antimicrobial effects against *Candida albicans*. (Cowan,1999 ;Rakotonirainy and Lavedrine,2005) reported that clove had antifungal activity and general antimicrobial activity . Also *Peganum harmala* showed antimicrobial activity against this fungi, these results agreed with other studies by (Bogdadi *et.al*,2007) who founded that *Peganum harmala* had high anticandidal activity with MIC ranging from (0.25-1mg/ml) .(Reza and Abbas,2007) founded that *Peganum harmala* have high antimicrobial activity against *Candida albicans* at MIC values ranged from (0.2-2.5) mg\ml.

The antimicrobial activity of these plant extracts may be due to presence of some active compound ex. Clove oil virtually pure eugenol (terpinoid) which can kill microorganisms by membrane distruption (Cowan,1999). *Peganum harmala* had harmane (alkaloid) the antibacterial activity attributed to their ability to intercalate with DNA (Phillipson & Neill 1987). Potassium alum makes attention to the water molecules from the cells and kill them by drying it .This result agreed with (Carpenter,2009) who founded that alum used for treating mouth ulcers by penetrate the membrane of the ulcer being drying it up

Table- 1-: MIC of plant extracts on growth of *Candida albicans* isolates that causes mouth lesion

Substances	**MIC mg/ml**					
	0.25	**0.5**	**1**	**2**	**3**	**4**
Peganum harmala	+	+	-	-	-	-
Syzygium aromaticum	+	-	-	-	-	-

(+): growth ,(-): no growth

Table- 2-: MIC of Potassium alum on growth of *Candida albicans* isolate that causes mouth lesion

substances	**MIC mg/ml**								
	2.5	**5**	**6**	**7**	**8**	**9**	**10**	**20**	**30**
Alum	+	+	+	+	+	+	+	-	-

Also these results showed that these plant extracts became more effective when used in combination and Peganum with Alum at the same plates. But when used alum combination with clove these antifungal activity of clove was decreased this may return to the chemical structure of these material which react together form chemical complex that inactive against these fungal isolates table-3-.

Table -3-: Synergism effect between MIC of Clove, Peganum harmala and alum on growth of *Candida albicans*

Substances mixture	**P.harmala +Clove**	**P.harmala +Alum**	**Clove +Alum**
Concentration mg/ml	**0.25+0.5**	**0.5+10**	**0.25+10**
growth	-	-	++

It can be concluded that P. harmala , clove or alum could probably be used for the control of these fungal isolates and prevent mouth infections such as oral thrush and mouth ulcer by

ISSN 2072-3875

washing the mouth by these substances or putting it on the lesions for some minutes and washing it with water.

References

Almuaamary A.G.,(2007) .Study of Identification Methods for candidiasis diseas. Ph.d Collage of Scientific . Myrath university.

Bogdadi H.A.A., Kokoska L., Havlik J., Kloucek P., Rada V., Vorisek K. (2007). In vitro Antimicrobial Activity of some Libyan Medicinal plant Extracts. J Pharmaceutical biology.,45(5): 386-391.

Balch and Balch, (2002) Prescription for Nutritional Healing, 2nd Edition, Avery Publishing Group, Garden City Park, New York.

Carpenter, B.L.(2009).Mouth ulcer:How to dry up mouth ulcers or canker sores with alum. Med. Microbiol.22:195-200

Cowan,M.M. (1999). Plant products as Antimicrobial Agents.J. Clin. Micribiol. Rev. 12(4):564-582.

Fu,Y., Chen,L., Shi,X., Wang,Z., Sun,S. and Efferth, T. (2007).Antimicrobial activity of clove and rosemary essential oils alone and in combination. J. Phyto. Res. 21(10):989-4.

Hughes WT., Flynn PM (2004). Candidiasis. In RD Feigin et al., eds., Textbook of Pediatric infectious Diseases, 5th ed., Vol 2, pp. 2569-2579. Philadelphia: Saunders.

Papas PG. (2004).IDSA Guidelines: Guidelines for treatment of candidiasis. Clinical Infectious Diseases, 38(2): 161-189. .

Phillipson, J. D., and Neill,M.J.(1987).New leads to the treatment of protozoal infections based on natural product molecules. Acta Pharm. Nord. 1:131-144.

Rakotonirainy, M.S.and Lavedrine, B. (2005).Screening for antifungal activity of essential oils and related compounds to control the biocontamination in libraries and archives storage areas. J. Interna. Biodeterio. & Biodeg. 55(2): 141-147.

Reza, V.R.M. and Abbas, H. (2007) Cytotoxicity and antimicrobial activity of alkaloids.. Pharmac. And Cytoto. 2(7): 677-680.

Sambrook J. (2004). Molecular cloning; a laboratory manual. Cold Spring Harbour Laboratory. New York.

ISSN 2072-3875