September 2015

IJESR Volume 3, Issue 9 ISSN: 2347-6532

Aushada-Trends in translational Research

A COMPARATIVE EVALUATION OF DIFFERENT MEDIA FOR THE DETERMINATION OF ANTICANDIDAL EFFICACY OF ESSENTIAL OILS

S.M. Shakira Begum[1], A.Anu Swedha *[2],Sumitha.J [2] Vijayalakshmi.K [3]

[1,2] Assistant Professor, Post Graduate Department of Applied Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India.

[3] Post Graduate Department of Applied Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India.

*Corresponding author:medmicroanu@gmail.com

ABSTRACT

In developing countries, infectious diseases present a serious public health issue and the burden is heightened by the economic crisis, high cost of industrialised medicines, inefficient public access to medical and pharmaceutical care. With the rise in the immunocompromised individuals due to advances in medical technology and pan epidemic of HIV infections, the number of invasive fungal infections has dramatically increased. Candidiasis is the commonest fungal disease found in humans affecting mucosa, skin, nails and internal organs of the body. Antifungals are generally used to treat fungal infections but these drugs are fewer and unusable due to antifungal drug resistance and toxicity, making people move towards herbal drugs which are safe. Medicinal and aromatic plants currently being used commercially are indigenous to different parts of the world from which the essential oils are obtained. Essential oils are known to possess antiseptic qualities that can be harnessed to obtain antifungals. Though a wide range of literature is available on anticandidal ability of medicinal plants and essential oils, there are only a few publications regarding the standardization of methods to study the antifungal efficacy of these oils/plant extracts. The present paper evaluates the different media available to study the anticandidal activity of essential oils.

KEYWORDS: infectious diseases, candidiasis, antifungal, essential oil, standardisation

Introduction

Pathogenic microorganisms known to cause life threatening infections throughout the world has increased and is one of the leading causes of mortality and morbidity in immunocompromised patients especially in a developing country like India (Ara N et al., 2009). Fungal infections in humans, particularly those involving the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries (Muschietti L. et al., 2005).

During the last several years, there has been an increase in the magnitude of fungal infections as a result of growth in the number of immunocompromised population such as patients with HIV/AIDS, cancer and organ transplant recipients. Yeasts of the genus Candida (*Candida albicans* and *Non-Candida albicans*) are the fungal agents most frequently involved in the etiology of infectious diseases in HIV affected population. Candidiasis range from superficial infections such as oral thrush and vaginitis to more complicated systemic conditions such as candidemia(Odds FC, 1994). Though there is an availability of wide range of antibacterial agents, there are only a few antifungal agents against Candida species which have undesirable side effects, or very toxic, produce recurrence, or leads to the development of resistance and are highly ineffective (Fan SR, 2008). Therefore, it is necessary to search for novel antifungal agents which are highly effective and have less/no toxic effects.

Plant extracts or plant derived compounds are most likely to provide a valuable alternative resource for the treatment of candidiasis. Essential oils are volatile hydrophobic liquids known to contain the active components from medicinal and aromatic plants. Various essential oils have been used medicinally at different periods in history for the cure of various ailments (Kalemba D and Kunicka A., 2003). Though various medicinal oils are utilized today to explore the ability to develop antifungal agents, there is still a necessity for standardization of protocols and various parameters to study the anticandidal activity of medicinal plants/essential oils. The present study was designed to compare and evaluate various media which are routinely used for the preliminary screening of antifungal medicinal extracts/ essential oils.

Materials and Methods:

I) Essential oils used for the study:

The essential oils were flower essential oils of *Jasminium sambac* (Arabian Jasmine), *Jasminium malabaricum* (wild jasmine), *Rosa damascene* (Damask Rose) , *Mimusops elengi* (Magilam) and *Pandanus odoratissimus* (Kewda). The essential oils were purchased from commercial sources. The essential oils were selected for the study based on ethnobotanical data (Abdoul Latif F *et al.*, 2010).

ii) Cultures used for the study:

The culture used for the study was *Candida albicans MTCC* 3017 obtained from Microbial type Culture collection of Institute of Microbial Technology, Chandigarh, India.

iii) Media used (Nayan R.Bhalodia and V.J.Shukla, 2011):

The following were the different media used:

Mueller Hinton Agar, Mueller Hinton agar supplemented with 2% glucose, Tryptone Yeast extract glucose agar, Potato dextrose agar, Mueller Hinton agar supplemented with methylene blue ,Sabouraud's Dextrose agar

iv) Method of Preliminary screening (Navarro et al., 1996; Ghaleb Adwan et al., 2012):

a) Agar disc diffusion assay: Discs about 6mm in diameter was impregnated with various essential oils at a concentration of 20µl. About 3 to 4 colonies of the culture was inoculated into sterile saline and the turbidity adjusted by comparing with 0.5 McFarland standard. A lawn culture of of the organism was made on the corresponding media. The prepared discs were placed on the plate. Positive control and negative controls were included. The plates were then incubated at 37°C for 24 hours. The inhibition zones around each disc both in the experiment and in the control were measured.

b) Agar well diffusion assay: About 3 to 4 colonies of the culture was inoculated into sterile saline and the turbidity adjusted by comparing with 0.5 McFarland standard. Wells were cut in the medium using a sterile cork borer. 20 µl volumes of the essential oils were added to the wells. Postive and negative controls were included. The plates were allowed to dry for 30 minutes and then incubated at 37°C for 24 hours. The inhibition zones around each disc both in the experiment and in the control were measured.

v) Testing of essential oil (Doren S.H. *et al.*, 2011):

As essential oils are volatile, insoluble in water and highly viscous, it is necessary to dissolve the essential oil in Tween 80 which is the most used surfactant. Therefore the media employed in all the above tests were prepared by incorporating 0.02% Tween 80 to produce reproducible and standard results.

Results:

TABLE 1: Comparison of Different Media for Anti Fungal Activity of Essential Oil at 37°C

Method of Assay: 1.Agar disc diffusion assay 2. Agar well diffusion assay

Temperature of incubation: 37˚c Organism tested: *C.Albicans MTCC*

Medium used	Disc diffusion method					Well diffusion method				
	J.sambac	J.malabaricum	R.damascena	M.elengi	P.odoratissimus	J.sambac	J.malabaricum	R.damascena	M.elengi	P.odoratissimus
1.Muller Hinton Agar	Zone of inhibition in mm									
	-	-	-	-	-	-	-	-	-	-
2.MHA with 2% glucose	55	10	55	-	-	-	26	-	-	-
3.sabouraud's dextrose agar	-	-	-	-	-	-	-	-	-	-
4.Potato dextrose agar	-	-	-	-	-	-	-	-	-	-
5.Trypton yeast extract glucose agar	-	35	25	-	40	-	20	22	25	30
6. Mueller Hinton Agar with methylene blue	-	-	-	-	-	-	-	-	-	-

For the comparative evaluation of different media using 5 different essential oils at a temperature of 37°C, using two different methods of screening, Mueller Hinton agar with 2% glucose and tryptone yeast extract glucose agar showed effective zone inhibition.

TABLE 1: Comparison of Different Media for Anti Fungal Activity of Essential Oil at 25°C

Method of Assay: 1. Agar disc diffusion assay 2. Agar well diffusion assayTemperature of incubation: 25˚c Organism tested:*C.Albicans MTCC*

Medium used	Disc diffusion method					Well diffusion method				
	J.sambac	J.malabaricum	R.damascena	M.elengi	P.odoratissimus	J.sambac	J.malabaricum	R.damascena	M.elengi	P.odoratissimus
1.Muller HintonMinter in Agar	Zone of inhibition in mm									
	-	-	-	-	-	-	-	-	50	42
2.MHA with 2% glucose	-	30	45	-	25	-	33	45	50	-
3.sabouraud's dextrose agar	15	-	-	15	11	-	-	43	30	14
4.Potato dextrose agar	23	20	-	-	11	-	45	-	48	-
5.Trypton yeast extract glucose agar	-	25	-	18	33	-	15	22	22	25
6. Muller HintenAgar with methylene blue	-	-	-	-	-	-	-	-	-	-

For the comparative evaluation of different media using 5 different essential oils at a temperature of 25°C, using two different methods of screening, Mueller Hinton agar with 2% glucose and tryptone yeast extract glucose agar showed effective zone inhibition followed by Potato dextrose agar and Sabouraud's Dextrose agar.

Discussion:

Worldwide infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. The resistance of pathogenic fungi including *Candida albicans* and non-*Candida albicans* species isolated from patients against

antifungal agents has increased. Based on the toxicity and low potency, combined with the increasing side effects of these drugs, novel fungal therapies with fewer side effects on humans are urgently required for effective management of candidiasis infections. These negative health trends call for a renewed interest on the strategies for treatment and prevention (Rekha.S and Vidyasagar.G.M, 2013)

Historically plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Essential oils and their chemical components are known to exert antimicrobial activity. Essential oils have cytophylactic, antiseptic, wound healing as well as anti-fungal and anti-inflammative properties (Pattnaik S *et al.*, 1996).

In the present study, the anticandidal efficacy of five flower essential oils were studied using six different media at two different temperatures. Two methods were used for preliminary screening. The selected oils have known to exhibit anticandidal activity. The method used to assess the anticandidal activity and the choice of media and test organisms varies between publications. A method frequently used to screen plant extracts/essential oils is the agar disc diffusion assay. The usefulness of this method is limited to the generation of preliminary qualitative data only, as the hydrophobic nature of essentials oils/plant extracts prevents the uniform diffusion of these substances through the agar medium. Another alternative method is the agar well diffusion method which is easier to perform with lucid results. Different media is used to study the anticandidal activity of essential oils but there is always the need for a standard, reproducible method for assessing essential oils.

Although CLSI (Clinical and Laboratory Standards Institute) methods have been developed for assessing conventional antimicrobial agents such as antibiotics, these methods with minor modifications such as use of different media and laboratory conditions can be made suitable and feasible for the testing of essential oils and plant extracts.

Conclusion:

Therefore to conclude, good results were observed with Mueller Hinton agar with 2% glucose and Tryptone yeast extract glucose agar followed by Potato dextrose agar and Sabouraud's Dextrose agar. Mueller Hinton agar and Mueller Hinton agar with methylene blue showed no results. Therefore, Mueller Hinton agar supplemented with 2% glucose and tryptone yeast extract glucose agar can be used as an alternative cost-effective and easily prepared media to study the anticandidal activity of medicinal plant extracts and medicinal oils. Maximum zones were exhibited

at a temperature of 25°C than 37°C which could be used as the incubation temperature to study and screen essential oils for effective productivity.

References:

1. Abdoul-Latif F, Edou P, Eba F, Mohamed N, Ali A, Djama S, Obame L, Bassolé I, Dicko M.,2010, Antimicrobial and antioxidant activities of essential oil and methanol extract of Jasminum sambac from Djibouti. Afr. J. Plant Sci., 4(3),pp 038-043
2. Ara N, Nur M.H, Amran . M, Wahid M.I, Ahmed M, 2009, *In vitro* antimicrobial and cytotoxic activities of leaves and flower extracts from *Lippiaalba*. Pakistan Journal of Biological Sciences; 12(1),pp 87-90
3. Doreen S.H. NG, Laili C. Rose, Hamdan Suhaimi, Habsah Mohamad, Mohd Z. H. Rozaini And Mariam Taib, 2011, Preliminary evaluation On The Antibacterial Activities Of Citrus Hystrix Oil Emulsions Stabilized By Tween 80 And Span 80.International Journal Of Pharmacy And Pharmaceutical Sciences; 3(2),pp 209-212
4. Fan S.R, Liu X.P, Li J.W.,2008, Clinical characteristics of vulvovaginal candidiasis and antifungal susceptibilities of Candida species isolates among patients in Southern China from 2003 to 2006. Journal of Obstetrics Gynaecology Research, 34(4), pp 561-566
5. Ghaleb Adwan, Yousef Salameh, and Ali Barakat, 2012, Assessment of antifungal activity of herbal and conventional toothpastes against clinical isolates of *Candida albicans.* Asian Pacific Journal of Tropical Biomedicine, 2(5),pp 375–379
6. Kalemba .D and A. Kunicka, 2003, Antibacterial and Antifungal Properties of Essential Oils. Current Medicinal Chemistry, 10(10), pp 813-829
7. Muschietti L, Derita M, Sülsen V, Muñoz J, Ferraro G, Zacchino S, Martino V.,2005, *In vitro* antifungal assay of traditional Argentine medicinal plants. Journal of Ethnopharmacology, 102,pp 233-238
8. Navarro V, Villarreal M.L, Rojas G and Lozoya X., 1996, Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. Journal of Ethanopharmacology, 53, pp 143-147
9. Nayan R.Bhalodia and V.J.Shukla., 2011, Antibacterial and antifungal activities from leaf extracts of *Cassia fistula L.*: An ethnomedicinal plant. Journal of Advanced Pharmaceutical Technology and Research, 2(2), pp 104–109
10. Odds F.C.,1994. Candida species and virulence. ASM News ;60, pp 313–318

11. Pattnaik. S ,Subramanyam V.R and Kole. C., 1996, Antibacterial and antifungal activity of ten essential oils *in vitro*. Microbios, 86(349), pp 237-46

12. Rekha Sharanappa and Vidyasagar G.M., 2013, Anti-Candida activity of Medicinal Plants. A Review. International Journal of Pharmacy and Pharmaceutical Sciences, 5(4), pp 9-16