Effect of Selected Plant Extracts on Malssezia Furfur in Culture

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Abstract: The affectivity of ethanol extracts of clove (Syzygium aromaticum), curcumin (Curcuma longa), ginger (Zingiber officinale) and khat (Catha edulis) were examined against Malassizia furfur. Ethanol extracts were prepared as (10% W/V). The yeast was obtained from ptyriasis versicolor (PV) infections and Sabouraud's dextrose agar medium + olive oil was used to isolate the yeast from the skin scrapes. The results of triplicate tests showed no yeasts developed in clove's extract treatment. The activity of curcumin extract was less than the medicinal soap treatment which was used as positive control. While ginger and khat treatments affect on the visible colonies characteristics only but not on their abundance in culture plates. The MIC for the clove extract and medicinal soap was compared by series of dilutions (10,5,2.5,1.25,o.625) mg/ml. Clove represent antifungal activity till the end, while it was 1.25mg/ml in medicinal soap treatment.

Keywords: Malassezia, Anti fungal, Clove, Curcumin, Ginger, Khat

1. Introduction

Mallasizia species are a part of human skin normal mycobiota beside a variety of homeothermic animals. They may cause several infections to skin and its appendices as opportunistic pathogen (Sugita et al 2010). /1. Cabans (2014) /2 listed 14 species of Malassezia cause diseases to human and animals. Among these, Malassezia furfur the main causative agents of pityriasis versicolor and dandruff (Chee and Lee 2009). /3. Other literatures maintained that M. globosa and M. restricta are the most frequent species associated with pityriasis versicolor (Sugita et al. 2001)/4, they are also the most common species on healthy human skim (Gaitanis, 2013) /5.

Although Malssezia causes a worldwide infections, these diseases showed a high incidence in tropical and sub tropical areas particularly in the poverty and poor sanitation sites, peoples live in these areas may have the infection yearly-around (American Academy of Dermatology).

Malassezia infections were treated by azole and its derivatives (Ngatu et al, 2011)/6 . In the same time, azole resistant Malassezia was reported (Dwivedi et al, 2010)/7, the side effects of such pharmaceutical drugs (Santhanam et al, 2014) /8 as well as their high costs incourag to searching for a new antifungal compounds. Several researches were carried out to evaluate the affectivity

of plants extracts against Malassezia (Mbakwem – Aniebo et al,2012/9; Santhanam et al,2014 /8; Ngatu et al, 2011/6; Nazeri et al, 2015/11; Tarazooie et al 2004 /10). The aim of this study is to evaluate and to compare the antifungal activity of four medicinal plants on Malassezia growth in vitro.

2. Materials and Methods

Malassezia isolates/ The yeast were isolated from two samples (farmer 25 years and a student with 20 years old). Skin scrapes were collected from neck with hyperpigmention symptom . The direct microscopic observation by KOH solution and lactophenol cotton blue stain was followed to recognize yeast structures in the specimens (Boekhout et al, 2010). The skin scrapes were cultured on Sabouraud's Dextrose agar medium, two ml of olive oil were added on the surface of each plate. Plates were incubated in 30oC ± 2.

Table (1): Plant materials / Four medicinal plants were selected

English name	Botanical name	Family	Processed part
Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome
Ginger	*Zingiber officinal*	Zingiberaceae	Rhizome
Catha	*Catha edulis*	Celastraceae	Terminal twigs
Clove	*Syzygium aromaticum*	Myrtaceae	Flower buds

Plants materials were brought form the local market, they were washed thoroughly, and were left in room temperature for seven days (complete dry). Tumeric and Ginger rhizome s were cut into pieces. The dried powder were obtained by crushing samples by electrical grinding machine, materials were kept in a glass jars with a fit cover for farther work.

Plant extractions/ To prepare the plant extractions, (50) gm of powder were dissolved in 5oo ml of ethanol 96% in soxhlet apparatus until color disappearing. The solution was dried by rotary evaporator in 37oC. This extract was considered as 100% , and was kept in refrigerator for farther use.

Antifungal test/ One gram from each antimycotic agents of the stock concentration was added to (99) ml of sterile SDA medium before solidify. Culture medium was poured in Petri dishes (99mm) then two ml of olive oil were added on the surface, inoculation was carried out after an hour. The inocula were spread on the SDA via sterile glass road. Triplicate for each agent as well as control treatments were followed. All treatments were incubated in 30o ±2, and the abundance of yeast's colonies in culture plates were recognized after seven days.

MIC determination/ To determine the minimum inhibition concentration(MIC) of clove extract and medicinal soap which represent the highest antimycotic activity. A serial dilutions from the original stock concentration (100%) were prepared. They were (10,5,2.5,1.25,o.625) mg/ml of Sabouraud's dextrose broth with olive oil. A Sadauraud's broth medium was used as a negative control treatment. A loop full from yeast culture was inoculated in each test tube. The treated culture as well as the negative control were incubated in 30 Co±2.The development of yeast in culture was emphasized by light microscopic examination.

3. Results and Discussion

Malassezia infections to human skin are commonly associated with high sebaceous secretion. In this study, the symptoms of hyperpegmintation were observed in both individuals easily. The infection was confirmed by direct microscopic exam for the skin scraping, yeast cells and other structures were recognized in microscopic field, it is a simple, easy ,and a quick method to emphasize on the causative agent of pyteriasis versicolor (Boekhout et al, 2010).Malassezia produce white creamy colonies with smooth texture (fig.1,C) on culture medium (SDA+ olive oil). The antifungal activity of ethanol extractions of the four selected medicinal plants exhibited variable results.

Clove extract was the best followed by medicinal soap, curcumin, ginger, and khat respectively. The studies related to using clove extraction against Malassezia are very rare. Clove (Syzygium aromaticum) extract had the highest efficacy among the other treatments used in the present study. Yeast colonies absolutely disappear from the plates containing culture medium of (SDA+ olive oil + clove extract 1%) (fig-1,D1).

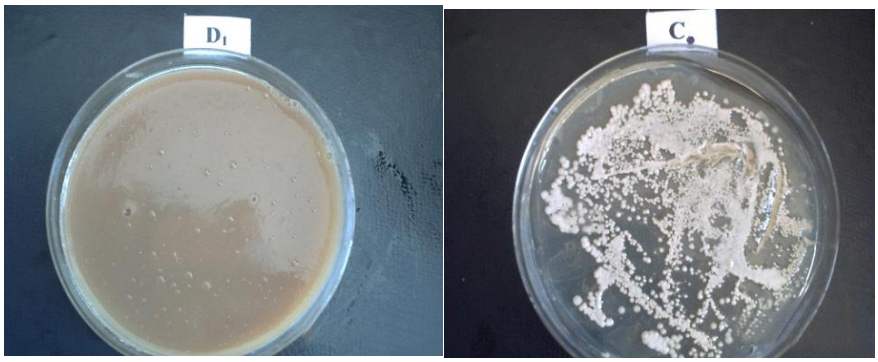

Figure (1): C-Malassezia growth on SDA. D- Malassezia growth on SDA+clove extract

Investigation on the antagonistic of clove oil against some common human fungal pathogen showed that the clove oil represented a strong affectivity (Rana et al, 2011). In a study to control food borne bacteria and fungi which cause food spoilage, clove oil showed high activity, it was more than the activity of standard chemical compound (sodium propionate), and also oil was found to be better than its extract as antagonistic agent (Gupta et al, 2008). The medicinal soap (fig.2, D2) affects on the colony morph as well as reduced the total number of colonies. Its activity may due to both, its chemical components and the ability to dissolving the olive oil.

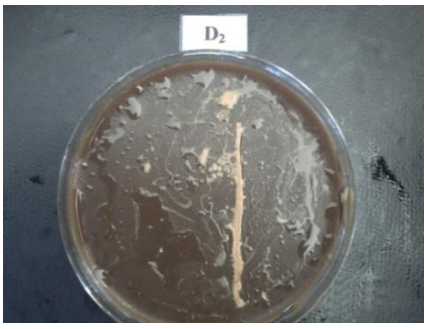

Figure (2): Malassezia growth on SDA+medicinal soap

Several studies have reported the broad-spectrum antimicrobial activity for curcumin (Curcuma

longa) (Moghadamtousi et al. 2014). In the present study, the ethanol extract of curcumin exhibit a reducing in colonies abundance (fig.3, D4). Ungphaiboon et al (2005) demonstrated that the methanol extract of turmeric had antifungal activity against Cryptococcus neoformans and Candida albicans, beside the dermatophytes.

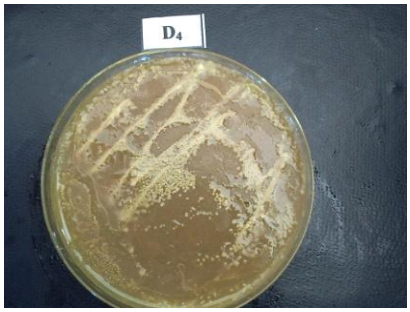

Figure (3): Malassezia growth on SDA+ curcomin extract

Ginger (Zingiber officinale) extracts showed a weak affectivity on colony abundance ,while there is an observed conversion in colonies morph.(fig.4, B2). The same result was reported by (Vijyakumur et al. 2006) when they examined the antifungal activity of aqueous ginger extracts within other 19 plants. From other side, Sharma et al. (2011) emphasized in their work that ginger oil had antifungal activity against M. furfur but less than clove oil.

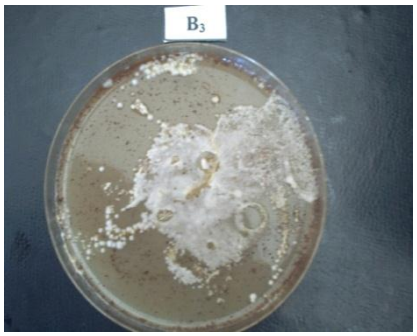

Figure (4): Malassezia growth on SDA+ ginger extract

Khat (Catha edulis) had no effect on visible colonies of M.furfur (fig.5,B3). Aqueous extracts exhibited potent antibacterial activity but not against yeast and molds which were tested by Siddiqui et al. (2012). The same result was recorded when the khat aqueous extract was tested against oral microorganisms (Al-hebshi et al. 2006).

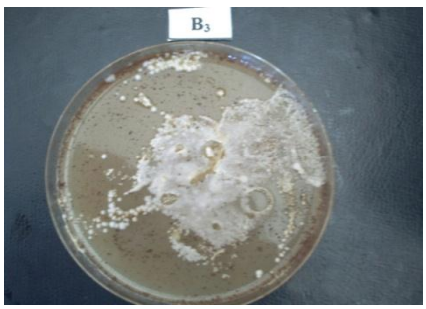

Figure (5): Malassezia growth on SDA+ khat extract

The MIC was estimated for the stronger antifungal materials only which include clove extract and medicinal soap. M.furfur did not grew in all concentrations of clove extract , while yeast cells were observed only in the lowest concentration (0.625mg/ml) of medicinal soap. According to the current study, we suggest clove (Syzygium aromaticum) for farther pharmaceutical and biochemical studies as well as in vivo tests to obtain an highly active drug in management pitryasis versicolor .

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