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RESEARCH ARTICLE

ANTIFUNGAL ACTIVITY OF APPLE CIDER VINEGAR AGAINST CLINICAL ISOLATES OF CANDIDA SPECIES

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ABSTRACT

Cutaneous fungal infections are common diseases in humans, and can also be caused by dermatophytic fungi and some yeasts. Superficial candidiasis is a common infection of the oral cavity, vagina, skin and esophagus, although most infections occur in patients who are immunocompromised. *Candida albicans*is responsible for many of these infections, but occasionally other members of the genus are associated, and generally infect the skin, nails, or mucous membranes. This study involves the determination of antifungal activity of Candida spp against apple cidar vinegar. The MIC of apple cider vinegar was appeared to be 0.25% for Candida spp**.** It is also a safer treatment modality with almost no side-effects. Apple cider vinegar seems to be the most economical product to have anti-fungal properties. Such activity may suggest promising effective alternative orsynergistic remedy to the popular antifungal drugs.

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INTRODUCTION

Cutaneous fungal infections are common diseases in humans, and can also be caused by dermatophytic fungi and some yeasts. Superficial candidiasis is a common infection of the oral cavity, vagina, skin and esophagus, although most infections occur in patients who are immunocompromised. *Candida albicans*is responsible for many of these infections, but occasionally other members of the genus are associated, and generally infect the skin, nails, or mucous membranes (Hall, 1999; Lacaz, 2002; Eggimann, 2003; Hay, 2005). The cause for vulvovaginal candidiasis is usually C. *albicans* affecting almost 95% of women followed by C. *glabrata* affecting 10-20% along with *C. tropicalis*and *C. krusei.* (Sobel, 2007; Develoux, 2007) . The candida species are known to be gram-positive yeast-like fungi that take up an oval or round shape. They can be isolated more commonly from primary endodontic infections, plaque, caries, subgingival microflora and active periodontal cavity. C.*albicans* are also a part of the normal oral microflora (Radeva, 2007) C. *albicans* is found to be the most common fungus involved in endodontic re-infections. The root canal is disinfected through mechanical preparation, chemical irrigation and placement of intracanal medicaments (Abbasi, 2015) .

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The conventional treatment of fungal disease is limited, and part of the reason is due to the limited spectrum of thecurrently antifungal drugs, and the expensive treatment,particularly due to the need of prolonged therapy. Thus, new drugs and alternative therapies are necessary, includingnatural products. (Silva, 2008). In recent studies, essential oils and extracts derived from natural plant species have been known to produce anti-microbial property against virulent genus of micro-organisms. Investigations concerning the evaluation of the biological activities of essential oils of some medicinal plantshave revealed that some of them exhibited antibacterial,antifungal and insecticidal properties (Burt, 2004). Because of the antimicrobial properties showed by essential oils, the aromatherapy has been used for treatment of serious skindiseases, in special, superficial mycoses (Carlini, 1986) Similarly, in this study the anti-fungal activity of apple cider vinegar was assessed. Apple cider vinegar (ACV) isa solution of acidic acid produced byfermentation of apples. Scientists have measured ninety different substances in apple cider vinegar such as thirteen types of carbolic acids, four aldehydes, twenty ketones, eighteen types of alcohols, eight ethyl acetates etc.It also contains important minerals, trace elements and vitamins (as listedunderneath) as well acetic acid, propionicacid, lactic acid and malic acid, enzymes, amino acids as well as roughage in theform of potash and apple pectin. (Jabir, 2011) ACV has various benefits like eliminating bad cholesterol, weight reduction, anti-aging and most

importantly has anti-fungal, anti-bacterial, anti-viral and anti-cancer effects. However, the FDA has not recognised ACV as a potent anti-microbial as there isn't scientific evidence for it. This study aims to create awareness among people about the various benefits of ACV, a household ingredient.

MATERIALS AND METHODS

CANDIDA ISOLATES

A total of 20 non-repetitive clinical isolates of *Candida species* were collected from different samples of immunocompromised individuals attending Saveetha Medical college, Thandalam. They were characterized by carbohydrate fermentation and assimilation tests and confirmed (Salkin, 2000). Isolates were preserved in semisolid Sabouraud chloramphenicol semi solid stock and stored at 4˚C until further use.

CHARACTERIZATION OF *CANDIDA* SPECIES

Candida species were further characterized by using Hichrom agar (Himedia, Mumbai).

Preparation of Hichrom agar

CHROMagar *Candida* (HiMedia, Mumbai) was prepared following manufacturer's instructions. About 21.02 gram of HiChrome *Candida* differentiation agar base (modified) was suspended in 500 ml of distilled water. It was heated to boiling gently to dissolve the medium completely. Then it was allowed to cool to 50^{o}C and rehydrated (one vial) contents of Hichrome *Candida* selective supplement was added under aseptic precautions. It was mixed well and poured into petri dishes. Isolates were identified on Hichrome agar based upon the characteristic color of the colony by sub culturing from Sabouraud's chloramphenicol agar plates and the *Candida* Hichrome plates were incubated at 37^{o}C for 24- 48 hours (Abbasi, 2015). Based on colour produced by the isolates speciation have been made.

Candida species	Colour
C.albicans	Green
C.tropicalis	Blue
C.krusei	Pink dry colonies
C.kefyr	Pale
C.parapsilosis	Pale

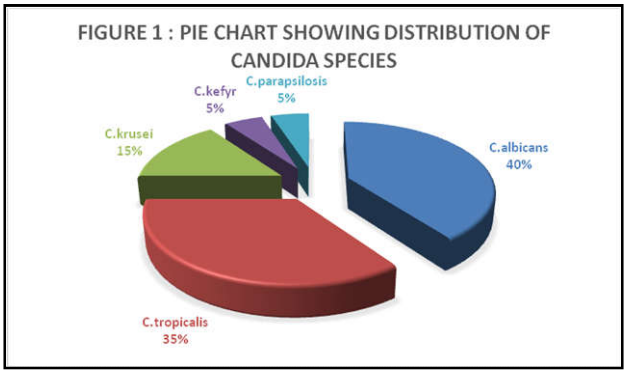
Detection of antifungal activity of apple cider vinegar against clinical isolates of Candida species

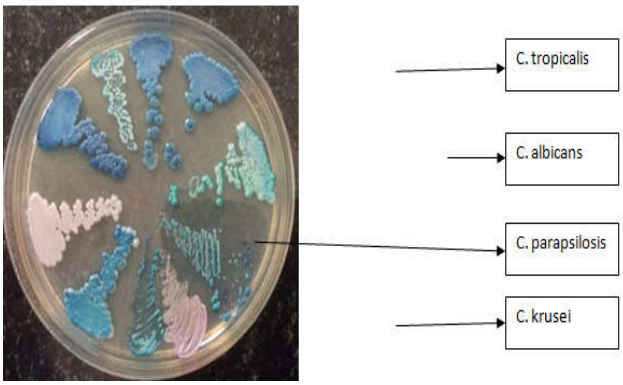
Antifungal activity of apple cider vinegar was tested against Candida isolates by minimum inhibitory concentration method. Agar dilution method was performed to attain the different concentrations of apple cider vinegar such as 0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1% and 2% SDA. Media containing various concentrations of vinegar were poured over the sterile petri dishes and allowed to dry. Media without vinegar was served as control plate. Spot inoculation of 0.5 McFarland standard turbidity adjusted isolates were made on the plates and incubated at 37ºC for overnight. The lowest concentration of the essential oils that completely inhibited the growth of isolates was considered as MIC.

RESULTS

Characterization of *Candida* species by Hichrom *Candida* agar

A total of 20 clinical isolates of *Candida* spp were seeded on to Hichrom *Candida* agar and results were tabulated based on pigment production.

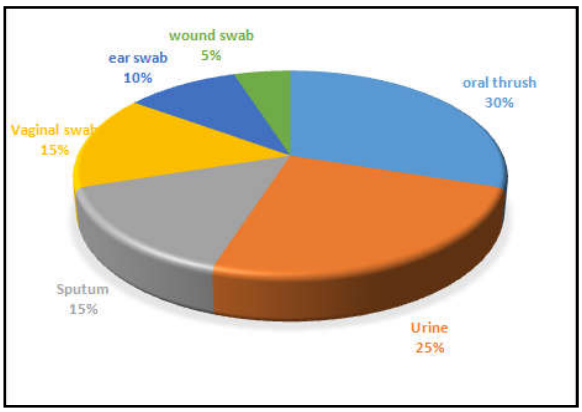




Representative picture showing *Candida* species on Hichrom *Candida* agar

Sample wise distribution of *Candida* species

Of the 20 clinical isolates of *Candida*spp, 6/20 (30%) were from oral thrush, 5/20 (25%) from urine, 3/20 (15%) from sputum, 3/20 (15%) from vaginal swab, 2/20 (10%) from ear swab and one (5%) from wound swab.

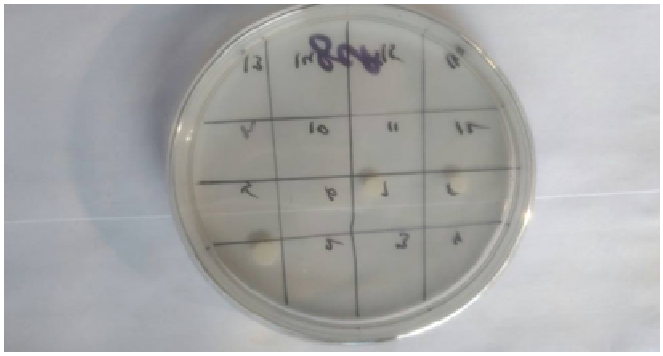


Pie chart showing the sample wise distribution of *Candida* species

Result of antifungal activity of apple cider vinegar against clinical isolates of Candida species

We have observed that, clinical isolates of Candida spp were inhibited from 0.25-0.5% of apple cider vinegar. The MIC of apple cider vinegar was appeared to be 0.25% for Candida spp.

Dilutions of apple cider vinegar	0.03%	0.06%	0.125%	0.25%	0.5%	1%	2%
No. of organisms	0	0	0	18 (90)	2 (10)	0	0



DISCUSSION

Vinegar, alone, has been used for cleaning and treating nail fungus, head lice, warts and ear infections. Consumers typically prefer natural preservative methods for inhibiting the growth of food borne pathogenic microorganisms in food. (Rauha *et al*., 2000) Several problems might be encountered with antifungal drugs, first; resistance; as fungi may become resistant to antifungal drugs due to target gene mutations, enzyme modification or to development of pump system that expels the drug out of thefungal cell (Finkel *et al*., 2005) second; toxicity; as antifungal drugs can cause systemic toxicities as hepatotoxicity and nephrotoxicity. Theseproblems necessitate searching for safer effective remedies with known antifungal activity (Jabir, 2011).

Apple cider vinegar is a commonly prescribed antifungal agent in folk medicine for treatment of fungal skin, earand vaginal infections. (Jabir, 2011) The antifungal activity of apple cider vinegar might be attributed to its malic acid, acetic acid contents or to other non-identified ingredients. The mechanism of inhibition fungi growth by organic acids is generally not considered a pH phenomenon. It is known that, growth and morphology of fungi are influenced by the pH of media (Higgins, 1999). Organic acids resulting a decreasing in pH value, this may influence the growth by acidifying the cell, which will consume a great amount of energy to maintain the intracellular pH homeostasis (Kang *et al*., 2003). Other explanations have also been proposed including the membrane disruption, the interruption of metabolic reactions, and the accumulation of toxic anions (Hassan *et al*., 2015).

The inhibition of microbial growth increases by lowering pH of the media, and most microorganisms are susceptible to antimicrobial effects in the presence of organic acids. This phenomenon is due to the hydrophobic feature of most organic acids, which allows free diffusion of the protonized form through cell membrane. This diffusion process takes place spontaneously due to pH and osmolarity gradients that exist between the inner and outer sides of the cell. The intracellular pH is higher than the extracellular, and the acid undergoes dissociation as soon as it enters the cytoplasm and then decreases the intracellular pH by releasing the proton. To counter the decrease of cytoplasmic pH, resulting from the ionization of the entered acid, the cell allocates the main part of its energy content to eliminate these newly formed protons which results in slower growth kinetics (Pelaez *et al*., 2012)
According to Hassan. R *et al*. (Pelaez *et al*., 2012), acetic acid shows strongest inhibition of fungal growth among other organic acids. Similarly, the study done by Prakasam *et al*. stated that natural products like aloe vera showed better inhibition when compared to calcium hydroxide. In this study, the MIC of apple cider vinegar against various isolates of Candida species appeared to be 0.25% for Candida spp**.** Anti-fungal activity is seen between 0.25% to 0.5% dilute concentrations of ACV. Thus, less than 1% of ACV is sufficient to produce anti-fungal effect against Candida.

Conclusion

Candida is one of the commonest opportunistic pathogen that causes disease in compromised hosts. (Nivedhaa Chezhian, 2015) Natural oils and products seem to be more efficient in their anti-fungal property when compared to anti-fungal drugs. It is also a safer treatment modality with almost no side-effects. Apple cider vinegar seems to be the most economical product to have anti-fungal properties. Such activity may suggest promising effective alternative or synergistic remedy to the popular antifungal drugs.

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