Research Article

Efficacy of different vinegars as antifungal agents against *Cryptococcus neoformans* and *Sarocladium kiliense*

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Abstract

Natural sources have caught the scientist's eye for the development of new active substances because of side effects of chemically synthetic preservatives and their ultimate hazardous effects on human health. In the present study, three types of vinegars; white distilled vinegar, grape vinegar and apple cider have been used to test the antifungal activity against *Cryptococcus neoformans* and *Sarocladium kiliense*. Rotten vegetables; cucumber and tomato were used to isolate these two fungal isolates. These isolates were identified using microscopic technique. Anti-fungal activity was observed followed by application of vinegars through agar well diffusion assay technique. All vinegars showed significant size of zone of inhibition against fungal specie. Distilled white vinegar showed the best effect against *C. neoformans* and *S. kiliense* forming zone of inhibition of 0.58 ± 0.05 mm and 0.6 ± 0.05 mm. Grape vinegar forms a zone of inhibition of 0.52 ± 0.05 mm and 0.48 ± 0.05 , while 0.4 ± 0.05 mm was observed against both fungal specie, as a result of application of apple cider. The results showed that vinegars can be used as an alternative to synthetic antifungal agents in order to overcome the drawbacks because they have active components i.e. phenolics and flavonoids etc. which have antifungal and anti-oxidant properties.

Keywords: Agar well diffusion; Anti-fungal agents; Apple cider; *Cryptococcus neoformans*; Distilled white vinegar; Grape Vinegar; *Sarocladium kiliense*

Introduction

Different antimicrobial agents have been in use for long time and these agents do not have any adverse effects. Many of the organic compounds are the antimicrobial metabolites of bacteria that are used to produce fermented foods. In fermentation process, raw materials are converted to products by the action of microorganisms and these products have acceptable qualities of food. Fermentation of food has a great economic value and these products have been contributed in improving the human health [1].

Scientific studies categorized the food borne pathogen behavior into two divisions, i.e., food science and medical field. Two different approaches are used to determine the behavior of food borne pathogens in food; commonly refers to as *in vitro* and *in situ*. *In vitro* studies aim at stimulating the behavior of food borne pathogen in laboratory giving a specific media for their growth, while *in situ* studies describe the microbial expression in real food under the conditions of storage and consumption of food [2].

C. neoformans contains two varieties namely *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans*. Colonies of *C. neoformans* are cream-colored and smooth, mucoid and have yeast like appearance of colonies. *C. neoformans* var. *grubii* is cosmopolitan in its distribution and this one cause 95% of all the *C. neoformans* infections. *C. neoformans* has been isolated from the menure of caged birds including canaries and parrots etc. *C. neoformans* var. *grubii* has also been isolated from the other environmental isolations including rotten or decomposed vegetables, fruits and fruit juices, wood dairy product and soil. The infections of *C. neoformans* var. *neoformans* are more prevalent in Europe including France, Italy and Denmark. Moreover, the infections are more related with older patients and may cause the skin infections [3]. The most infections associated to *Cryptococcus* are caused by the *C. neoformans* var. *neoformans* in humans [4]. *C. neoformans* is accountable for serious infections in patients with cellular immune defects, particularly patients with AIDS [5]. *Sarocladium* was formerly called *Acremonium*, but after study the taxonomy of *Acremonium*, some medically important species have been transferred to *Sarocladium*; i.e. *S. kiliense* (formerly *A. kiliense*) and *S. strictum* (formerly *A. strictum*). Morphologically these genera are similar, but they are phylogenetically distant. *Sarocladium* can be morphologically differentiated from *Acremonium* by its elongated phialides rising solitary on vegetative hyphae or on conidiophores that are sparsely or repeatedly branched, the production of abundant adelophialides and elongated conidia [6].

Different natural antimicrobials from the plant origin contain secondary metabolites having ability to inhibit or reduce the growth of bacteria, yeast and molds [7]. Plant essential oils have also been used as a flavoring agent in addition to its

antimicrobial activity [8]. A large number of antimicrobial agents originated from animal origin and involved in host defense mechanisms [9]. Among the different natural products, vinegars are also contributing in its antimicrobial properties [10]. Vinegars i.e. Apple cider has an important element; maleic acid with bactericidal and fungicidal activity. Maleic acid has been reported for the treatment of various infections [11].

Material and methods

Isolation and culturing of fungus

The microorganisms to be isolated; were grown on the Potato dextrose agar media (containing 3.9g PDA in 100ml water). Rotten vegetables; cucumber and tomato were used to isolate the fungal specie. Small pieces of these were inoculated in the center of two different PDA plates. Then the plates were incubated at 37°C for 72 hours.

Identification of fungal isolates

After incubation period, two different fungal isolates were obtained. They were identified by microscopic technique. Fungal smear of each species was made and then stained with crystal violet stain. The slides were then observed under microscope.

Agar-well diffusion method

Principle: The antimicrobials present in the natural antifungal (vinegars) were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition were mostly circular in shape as there was a confluent lawn of growth. The diameter of zone of inhibition was measured in millimeters.

Method to apply vinegars

PDA plates were prepared under aseptic conditions. Then, by using the borer, wells were made in the center of each plate. Three of the plates were swabbed with each of the two fungal isolates, using sterile cotton swabs. Then, 100 µl of each vinegar i.e. apple cider, distilled white vinegar and grape vinegar were added in the wells. The plates were then incubated for 72 hours at 37°C. The

vinegars were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. After incubation period, zones of inhibition were observed in each plate. The diameter of zone of inhibition was measured in millimeters with the help of Vernier caliper and diameter of each zone of inhibition was measured from three different directions. The mean values of zone of inhibition are mentioned in results.

Results

In present study, two different fungal species were isolated and identified on the basis of their colony morphological characteristics, color charts and microscopic examination. The isolated fungal species were *C. neoformans* and *S. kiliense*.

Morphological examination of fungal isolates (Fig. 1).

Microscopic examination of fungal isolates (Fig. 2).

Effect of distilled white vinegar on *C. neoformans* (Fig. 3).

Effect of grape vinegar on *C. neoformans* (Fig. 4).

Effect of apple cider on *C. neoformans* (Fig. 5).

Effect of distilled white vinegar on *S. kiliense* (Fig. 6).

Effect of grape vinegar on *S. kiliense* (Fig. 7).

Effect of apple cider on *S. kiliense* (Fig. 8).

The best effect of distilled white vinegar against *C. neoformans* with mean value of 0.58 ± 0.05 mm (Fig. 9).

The zones of inhibition of vinegars against *S. kiliense* (Fig. 10).

The varying zone of inhibition against fungal species (Table 1).

Figure 1. (a) Colony of *C. neoformans* (b) Colony of *S. kiliense*

Figure 2 (a) Microscopic examination of *C. neoformans* (b) Microscopic examination of *S. kiliense*

Figure 3. Effect of white vinegar on *C. neoformans* after 72 hours. Distilled white showed the activity against *C. neoformans* by forming zone of inhibition of 0.58 ± 0.05 mm after 72 hours

Figure 4. Effect of grape vinegar on *C. neoformans* after 72 hours. Grape vinegar showed the activity against *C. neoformans* by forming zone of inhibition of 0.52 ± 0.05 mm after 72 hours

Figure 5. Effect of apple cider on *C. neoformans* after 72 hours. Apple cider vinegar shows activity against *C. neoformans* by forming zone of inhibition of 0.4 ± 0.05 mm after 72 hours

Figure 6. White vinegar showed the activity against *S. kilense* by forming zone of inhibition of 0.6 ± 0.05 mm after 72 hours

Figure 7. Grape vinegar showed activity against *S. kilense* by forming zone of inhibition of 0.48 ± 0.05 mm after 72 hours

Figure 8. Apple cider vinegar showed activity against *S. kilense* by forming zone of inhibition of 0.4 ± 0.05 mm after 72 hours

Figure 9. Shows the best effect of distilled white vinegar against *C. neoformans* with mean value of 0.58 ± 0.05 mm. The moderate effect was observed for grape and least for apple cider with the mean value of 0.4 ± 0.05 mm

Figure 10. showing the zones of inhibition of vinegars against *S. kiliense*. Distilled white has the best effect against fungal species showing zone of inhibition of 0.6 ± 0.05 mm

Table 1. Showing the varying zone of inhibition against fungal species

Vinegars showing zone of inhibition (mm)			
Fungal species	Distilled white	Grape	Apple cider
<i>C. neoformans</i>	0.58 ± 0.05	0.52 ± 0.05	0.4 ± 0.05
<i>S. kiliense</i>	0.6 ± 0.05	0.48 ± 0.05	0.4 ± 0.05

Discussion

Vinegars have been used as antifungals from ancient times to preserve food from fungal and bacterial pathogens. The key component in vinegar is acetic acid which has antifungal activity. The use of vinegars to cure infections is reported since the time of Hippocrates. A combination of honey and vinegar was also used to treat cough in ancient times. This study is also conducted to evaluate antifungal activity of different types of vinegars. Vinegars are used against fungus in various applications which has been demonstrated to prevent the germination of conidia of vegetable decaying fungi. Vinegar has showed antifungal properties against conidia of fungi growing on fruits. Hence it is a known antimicrobial agent and there is evidence to suggest that it has some antifungal properties [12].

In the present study, *C. neoformans* and *S. kiliense* have been isolated from vegetable source i.e. rotten cucumber and tomato. Three types of vinegars named distilled white vinegar, grape vinegar and apple cider have been utilized against the growth of fungus to test their antifungal potential. Vinegars showed significant zone of inhibition of different diameters against fungal species indicating their effectiveness.

Our results showed prominent zones of inhibition against the fungal species meaning these vinegars have best antifungal activities. Among all vinegars, white distilled vinegar has better antifungal activity against both fungal isolates i.e. *C. neoformans* and *S. kiliense*. While the grape vinegar and apple cider also showed activities against fungal isolates.

No literature has been found in which apple cider, grape vinegar and white synthetic vinegar have applied against fungal species e.g. *C. neoformans* and *S. kiliense*.

Conclusion

Although, traditionally vinegars have been used as food flavoring and preservative

agents, but recent studies have shown that the active components present in vinegars are beneficial for human health as well e.g. maleic acid, phenolics and flavonoids are antifungals as well as antioxidants. In the present study, vinegars are used against fungi namely *C. neoformans* and *S. kiliense*. The resultant antifungal activity of vinegars showed that they can be used as an alternative to synthetic antifungal agents. Phenolic compounds in vinegar can scavenge superoxide anion and free radicals *in vivo* resulting in a potent antioxidant activity.

Authors' contributions

Conceived and designed the experiments: A Imtiaz & MA Khan, Performed the experiments: H Niamat & Muneeba, Analyzed the data: A Imtiaz & H Niamat, Contributed materials/ analysis/ tools: Department of Biology, Lahore Garrison University, Lahore, Wrote the paper: H Niamat & A Imtiaz.

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