

Research Article

Activity of Some Disinfectants, Detergents and Essential Oils on Growth of the yeast *Candida albicans*

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Abstract

The study was conducted for estimating antifungal activity against *Candida albicans* of ten essential oil, garlic and onion juice, eight disinfectants and detergents by using agar diffusion well method.

The results showed high activity of clove oil, onion juice 50%, thyme oil, hydrogen peroxide (H_2O_2), lugol's iodine and detol for all the five concentration, sodium chloride (NaCl) and sodium carbonate (Na_2CO_3) at 5%, while the effect of apple cider vinegar were at 60% and 80%. Sodium hypochlorite showed moderate activity at all concentration. The result of combination between clove oil and coconut oil led to synergistic effect while the combination between each of (1, 2, 3 % H_2O_2 with each of apple cider vinegar and NaCl), (sodium chloride with apple cider vinegar) and (pumpkin oil with clove oil) lead to antagonism as well as the same results were reported when apple cider vinegar mixed with each of (thyme oil) and (clove oil with coconut oil). On the other hand, there is no synergistic or antagonistic effect of combination between 4-5% of H_2O_2 with apple cider vinegar and NaCl solution to the growth of *C. albicans*.

It was concluded that solitary use of compounds (clove oil, onion juice, thyme oil, H_2O_2 , NaCl, lugol's iodine, detol, and apple cider vinegar) was associated with high antifungal response regarding *C. albicans*; efficacy was reduced when used in combination. In exception to above finding synergistic effect was identified when a combination between clove oil and coconut oil.

Keywords: *Candida albicans*, plant oils, hydrogen peroxide, apple cider vinegar, detol, lugol's iodine, synergism, antagonism.

الخلاصة

اجريت الدراسة لقياس تأثير فعالية عشرة من الزيوت الأساسية، عصير البصل والثوم وثمانية أنواع من المنظفات والمطهرات على نمو الخميرة *C. albicans* بطريقة الانتشار بالهلام. أظهرت النتائج فعالية عالية لكل من زيت القرنفل، زيت الزعتر، عصير البصل 50%، والتركيز الخمسة المستعملة لكل من الديتول، بيروكسيد الهيدروجين (H_2O_2) والايودين. وتأثير كل من محلول كلوريد الصوديوم (NaCl) وكربونات الصوديوم (Na_2CO_3) عند التركيز 5% وخل التفاح عند التركيزين 60-80% وفعالية متوسطة لمنظف القاصر للتركيز المستعملة.

ونتائج الخلط بين زيت القرنفل وجوز الهند أدت الى فعالية تأزيرية بينما كانت نتيجة الخلط بين كل من (بيروكسيد الهيدروجين (H_2O_2) عند التركيزين 1، 2، 3% مع كل من خل التفاح ومحلول كلوريد الصوديوم)، (خل التفاح مع محلول كلوريد الصوديوم)، (زيت قرنفل مع زيت بذور القرع) أدت الى فعالية تضادية وكذلك أظهرت نتائج الدراسة عند خلط خل التفاح مع كل من زيت الزعتر و (زيت قرنفل مع زيت جوز الهند) أدت الى فعالية تضادية ومن جهة أخرى لم يظهر أي تأثير تأزيري أو تضادي عند خلط التركيزات 4-5% من بيروكسيد هيدروجين (H_2O_2) مع كل من خل التفاح ومحلول كلوريد الصوديوم على نمو *C. albicans*.

تم الاستنتاج بان الاستعمال المنفرد للمواد (زيت القرنفل، عصير البصل، زيت الزعتر، بيروكسيد الهيدروجين، كلوريد الصوديوم، الايودين، الديتول، خل التفاح) كانت لها فعالية عالية ضد المبيضة البيضاء، يقل التأثير عند الخلط بينهم ماعدا الخلط بين زيت القرنفل وزيت جوز الهند تؤدي الى فعالية تأزيرية.

Introduction

Candida albicans is a normal flora in gastrointestinal tract, oral cavity and vagina. Candidiasis can occur due to many causes i.e.

cutaneous, vaginal, oral, and systemic candidiasis. Systemic candidiasis associated with high mortality 33% to 54% and high morbidity in nose patients who survive [1]. The fourth most frequent cause of nosocomial bloodstream



infections was systemic candidiasis in the last decade. [2]. Candidiasis of mucous membranes and skin are the commonest types of candidiasis [3]. There is increasing need for new and universal antifungal drugs, which should be less toxic and more effective than those which are available.

Iodine is rapid fungicidal, bactericidal, virucidal and sporicidal effects because iodine is halogen releasing agent which exhibits its action by attacking nucleotides, amino acids, fatty acids and inhibition of DNA synthesis [5]. Hydrogen peroxide is an oxidizing agent used for effective control of different bacteria (especially gram-positive), their yeasts, spores and viruses [6], both compounds are common antiseptics and disinfectants in topical skin therapy [7], wound healing [5][8], preparation of preoperative sites [9], control of gingival plaques [10]. Apple cider vinegar can be used to inhibit and control candidal overgrowth [11].

Sodium hypochlorite (NaOCl) is widely used products for disinfection, antiseptic purposes and also for decontaminating non-critical surfaces with blood spillage in health care settings [12]. Antibiotic resistance is a great problem through the world, although new antibiotics are available [14]. However, laboratory data show the possibility of the relationship between antibiotic resistance and the use of disinfectants and antiseptics, so there is worldwide increasing concern regarding uncontrolled use of disinfectants and antiseptics [13]. This link between antibiotic resistance and the use of disinfectants and antiseptics may be due to that these agents have no specific mechanism of action, broad spectrum of activity and multiple targets [14]. The pollution of water bodies in hospitals are caused by environmental impact of successive usage of detergents [15].

Phyto-constituents of natural products also have biological activity that exhibits these actions by increasing membrane permeability leading to cell death owing to exposure to essential oils, which are liposoluble [16]. The essential oils (EOs) from many plants, although have limited information on their activity against human fungal pathogens [17], it is well known that they have antibacterial and antifungal activity [18]. Thyme oil is useful for asthma, bronchitis and respiratory tract infections [19]. The clove oil has beneficial analgesic, antiseptic, and anesthetic

effects [20] and worldwide used in dental medicine. Fungicidal activities of clove oil against yeasts are reported in previous studies [21], and human pathogenic fungi [22]. The study aimed to estimate the fungicidal activity of some disinfectants and plant oils and to know the effect of combination between them at low concentration on growth of *Candida albicans*.

Materials and Methods

Media Preparation

Sabouraud Dextrose Agar with chloram-phenicol and Muller-Hinton agar prepared according to the manufactured company (Himedia India) by complete dissolving the medium by heated it until boiling then sterilized in an autoclave for 15 minutes at 15lbs. Pressure (121°C). Chloram-phenicol (0.05g/L) added after autoclaving and cooling the media to 50°C. Any solutions which used for this study were sterilized by filtration by Millipore filter with diameter 0.22µm.

C. albicans Collection and Identification

The clinical *Candida* isolates were collected and identified in Azadi teaching hospital and re-identified in the medical laboratory techniques department. Identification depended on colony characters appeared as white to cream soft and smooth with fermentation odor, gram stain showed gram positive with large cocci oval shaped cells with budding. Germ tube test was performed by a slightly inoculated 0.5ml of human serum with test yeast and incubated at 37°C for 1-3 hours; the production of germ tubes by the cells is diagnostic for positive result. Confirmatory identification done by API *Candida* (BioMérieux-France) that give the code number (7112) [23][24].

Detergent and Disinfectant Solutions Preparation

To find the accurate percentage of apple cider vinegar and detol approximately we considered vinegar is absolute and detol which prepared as volumes (1 volume of detol with 4 volumes of distill water considered 1%, 2 volumes of detol with 3 volume of distill water was 2%, 3 volumes of detol with 2 volumes of distill water was 3%, 4 volumes of detol with 1 volume water was 4%, whole detol which concentration 5%). Onion and garlic juice was prepared from fresh origin. Table 1, shows the types of disinfectants,

detergents and herbs oils used for this study with their concentration, and Manufacturer Company and country.

Essential Oil Preparation

Essential oil either tested solely (not diluted neither mixed as provided by manufacture company) or combination made between two types of essential oil by mixing equal volume of each type (v/v) or between two essential oil with equal volume of prepared concentration of apple cider vinegar (for example: 1m of essential NO.1 + 1ml. of essential NO. 2 + 1ml. of apple cider vinegar with concentration 20 or 40%) in tubes then added to wells.

C. albicans Suspension Preparation

Suspension of the yeast prepared by transferred 1 to 3 colony from culture aged 18-24 hours, to a test tube contained 5ml sterilized normal saline

compared and adjusted with the tube No. (0.5) of McFarland (commercially available) standard which gives a cell density 1.5×10^8 cell/ml., a sterile cotton swab was dipped into the suspension and then swabbed evenly across the surface of a Muller-Hinton agar plate; the plates were incubated at 37°C for 30 minutes.

Activity Assay by Agar Diffusion Well Method

From each prepared diluted concentration (see Table 1), detergent, disinfectant, and prepared essential oil 100µL added to each of the wells (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). The plates were incubated (not converted) for 24h at $36^\circ\text{C} \pm 1^\circ\text{C}$, under aerobic conditions. Confluent yeast growth was observed after incubation. Inhibition of the growth was measured in mm. Tests were performed in duplicate [25].

Table 1: Disinfectants, detergents, and plant oils are used in this study.

Source	Company name/product	Concentration used
Hydrogen peroxide (absolute)pH=4.9	Schailau company/Indian	1%, 2%, 3%, 4%, 5%
Sodium hypochlorite 6% pH=14	FAS/Iraqi	0.25%, 0.5%, 0.75%, 1%, 1, 25%
Sodium chloride (NaCl) powder	Zer /Turkish	1%, 2%, 3%, 4%, 5%
Apple cider vinegar PH=2	Zer /Turkish	1%, 2%, 3%, 4%, 5%, 20%, 40%, 60%, 80%, 100%
Lugol's Iodine 5%	Eisen-Golden/Laboratories/USA	1%, 2%, 3%, 4%, 5%
Detol (Lenol) hloroxyleneol, pine oil, unknown conc. PH=8	Spartan /Jordan	1%, 2%, 3%, 4%, 5%
Sodium carbonate-poweder	Turkish product	1%, 2%, 3%, 4%, 5%
Detergent (Altunsa)	Altunsa company/Turkish product	0.05%, 0.15%, 0.25%, 0.35%, 0.45%
Coconut oil	Hemani International/Keps/Pakistan	Whole oil
Clove oil	Hemani International/Keps/Pakistan	Whole oil
Pumpkin oil	Emad factory for herbs plant oil/Iraqi	Whole oil
Castor oil	Emad factory for herbs plant oil/Iraqi	Whole oil
Olive oil, first cold press)	Al-Wazir –company /Lebanon	Whole oil
Garlic juice	Prepared from fresh Garlic	50% (1 v+1v distill water)

Onion juice	Prepared from fresh onion	50% (1 v+1v distill water)
Pine nut oil	Hemani International Keps/Pakistan	Whole oil
Rosemary oil	Emad factory for herbs plant oil/Iraqi	Whole oil
Thyme oil	Hemani International Keps/Pakistan	Whole oil
Tannins Oil	Emad factory for herbs plant oil/Iraqi	Whole oil

Results and Discussion

C. albicans live as harmless commensals in the gastrointestinal and genitourinary tract. Overgrowth of these organisms leads to disease [26]. More than three fourth of women will suffer from a *C. albicans* infection, usually vulvovaginal candidiasis, in their lifetimes [27]. Therefore, the study used different disinfectants and detergents to estimate the best efficacy at low concentration for each one.

Table 2 showed that *C. albicans* was resistant to concentration 1%-4% for both sodium chloride and sodium bicarbonate while showed sensitivity at 5%. These results agreed on the results of Cintia *et al.* [28] who confirmed that sodium bicarbonate at concentration 1% did not have antimicrobial activity against *C. albicans* while Sousa *et al.* [29] proved the efficacy of higher

concentration of this solution in reducing biofilm formation equivalent to 5% compared to 1% in this study. Another study revealed that higher concentration of sodium bicarbonate solution possesses an inhibitory effect against oral candidiasis [30]. The results of Aiman and Fahad [31] showed sodium chloride affects *C. albicans* only in concentrations higher than 1g/20ml when samples are immersed for at least eight hours this result is same as our result and incompatible with Basson *et al.* [32] who showed that the use of 0.66% of NaCl was significantly effective in reducing viable microorganisms compared to tap water [32]. Sodium bicarbonate has been reported to be virucidal and inhibit the growth of several fungi, but its mechanism of action is unclear [33].

Table 2: Effect of disinfectants and detergents on growth of *C. albicans*

Disinfectants and detergents	Concentration	1 %	2%	3%	4%	5%
	Zone of inhibition in millimeter					
Apple cider vinegar	Re	Re	Re	Re	Re	Re
Sodium chloride (NaCl)	Re	Re	Re	Re	Re	40
Peroxide hydrogen (H ₂ O ₂)	40	40	40	40	40	40
Lugol's Iodine	40	40	40	40	40	40
Sodium bicarbonate	Re	Re	Re	Re	Re	22
Detol	40	40	40	40	40	40

*Re= resistant (no inhibition zone)

C. albicans appeared resistance for all selected concentration (Table 3) of each altunnsa detergent and apple cider vinegar this mean *C. albicans* has ability to respond to extracellular pH [34] but when increase the concentration, apple cider vinegar demonstrate antifungal activity at 60% and 80% then decrease the sensitivity when used absolute apple cider vinegar. Such results are compatible with a study showed that the concentration (60.5ml/100ml) vinegar was effective in when samples were immersed for one hour [31], while incompatible with study of Hayder *et al.* [35] who reported

that apple cider vinegar (5%) has significant antifungal effect against *C. albicans* and Non-*C. albicans* when compared with the negative control. White vinegar was found to be inefficient in reduction of the number of *C. albicans* colonies that adhered to specimens [29], however Peker *et al.* [36] have reported that white vinegar was the most effective disinfecting agent for tested microorganisms and acrylic resins.

C. albicans revealed full sensitivity of each of hydrogen peroxide, detol and lugol's iodine (Table 2) at all concentration 1%-5%. The

fungicidal effects of iodine exhibited by targeting amino acids, fatty acids, nucleotides and by inhibition of DNA synthesis [6][8]. Hydrogen peroxide efficient on controlling yeasts owing to free OH-radical's formations that oxidize thiol-groups of lipids, proteins and DNA breaking [6]. This result is agreed with Bryan [37] who believed that hydrogen peroxide could be fungicidal. Sodium hypochlorite has antifungal activity at five concentrations with sensitivity at 1% and 1.25%. Therefore, the present study corroborates the findings that illustrated 1% sodium hypochlorite has antifungal activity when used with *C. albicans* [28][38].

The results in Table 4 and 5 showed a combination between of 1-3% H₂O₂ with 1-5% of apple cider vinegar lead to antagonism and the inhibition not affected when concentration of H₂O₂ at 4%-5%. While the combination of 1-4%

H₂O₂ with 1-5% NaCl solution lead to antagonism and no effect of combination at 5% H₂O₂. Peracetic acid is the resulting chemical when vinegar and hydrogen peroxide are mixed together in one container, and this product may cause some reaction to those who are very sensitive to it, while if you reacted sodium chloride with hydrogen peroxide, the only possible products in aqueous solution would be sodium hydroxide and chlorine gas [39], which is alkaline environment which overgrowth of *Candida sp.* The results in Table 6 illustrate the combination between apple cider vinegar and NaCl solution lead to negative effect on growth of *C. albicans*. This may be NaCl combines with acetic acid from the vinegar producing sodium acetate but the mechanism that leads to overgrowth was not understood.

Table 3: Effect of detergent, sodium hypochlorite and apple cider vinegar on growth of *C. albicans* at selected concentrations.

Altunsa concentration (gram/100ml)	Inhibition zone (mm)	Sodium hypochlorite concentration (v/100ml)	Inhibition zone (mm)	Apple cider vinegar concentration (v/100ml)	Inhibition zone (mm)
0.05%	Re	0.25%	22	20%	Re
0.15%	Re	0.5%	20	40%	Re
0.25%	Re	0.75%	20	60%	40
0.35%	Re	1%	30	80%	40
0.45%	Re	1.25%	32	100%	10

* Re= resistant (no inhibition zone)

Table 4: Effect of the combination between apple cider vinegar with H₂O₂ on growth of *C. albicans*.

Concentration Apple cider vinegar	Concentration of H ₂ O ₂				
	1%	2%	3%	4%	5%
Zone of inhibition in millimeter					
1%	11	20	25	31	30
2%	12	20	30	34	36
3%	12	24	25	40	30
4%	13	21	25	40	40
5%	13	23	25	40	40

* R= resistant (no inhibition zone)

Table 5: Effect of combination between NaCl solutions with H₂O₂ on growth Of *C. albicans*.

Concentration of Sodium chloride (NaCl)	Concentration of H ₂ O ₂				
	1%	2%	3%	4%	5%
Zone of inhibition in millimeter					
1%	16	18	22	31	40
2%	17	19	22	20	40
3%	12	12	25	31	40



4%	15	17	25	30	40
5%	17	20	25	30	40

* R= resistant (no inhibition zone)

Table 6: Combination between apple cider vinegar with NaCl solution at selected concentration

Concentration (NaCl solution+ apple cider vinegar)	Zone of inhibition in millimeter
1%+20%	Re
2%+40%	Re
3%+60%	Re
4%+80%	Re
5%+100%	Re

* Re= resistant (no inhibition zone)

The results in (Table 7) appeared that coconut oil, olive oil, castor oil, sweet almond oil, pumpkin oil, tannins oil and pine oil showed resistance against *C. albicans* while clove oil, thyme oil and onion juice appeared full sensitivity. Our study revealed antifungal activity of clove oil is in agreement with the data obtained by previous studies [20, 40, and 41]. These reports provide further support for treatment of mucocutaneous infection by eugenol's potential [42].

Thyme oil appears antibacterial activity which is useful in dental practice [19]. Thymol is a component of thyme which play role in growth inhibition of oral pathogens and can reduce dental caries by mixing with other essential oils [43]. Clove and thyme oil at different concentrations of the extracts have activity toward *C. albicans* [44][45]. In another study, antimicrobial susceptibility in order of sequence for thyme oil was *C. albicans* with minimum inhibitory concentration 16µl/ml [46].

The essential oil rosmarini officinalis showed anti-adherence and antifungal effects against *C. albicans* strands [16], it is confirmed the inhibitory effect at a concentration of 0.56 mg/ml, similar to nystatin that are reported from the authors therefore, the results of the present study corroborate the literature in confirming the antifungal activity of the essential oil rosmarini officinalis [16]. The high concentration of terpene in essential oil based on rosmarini officinalis is confirmed the fungicide and bactericide action of it against *C. albicans* strands. Terpene has ability of reducing cell growth by enzymatic inhibition and increase cell permeability which results microbial death [47]. It is well known the worldwide use of onion and garlic as a flavoring agent, onion and garlic also known to have medicinal properties. Onion bulbs

contain quercetin and glycosides; tannin, pectin, and alcohol extract show antileptazol and hypotensive analgesic properties. Onion extracts shows antibacterial properties. Garlic bulbs contain garlicin, volatile oils, pectin, allin and allistatins I and II. Alcoholic extract of garlic shows hypoglycaemic, antibiotic, bactericidal and fungicidal activities. The extract showed antileptazol, hypotensive and analgesic sedative properties [48]. The result of study showed resistance of *C. albicans* to garlic juice which is incompatible with Hamza [49] who reported Garlic & Onion extract individually (20mm) inhibition zone to each one against *C. albicans*. Clove oil is fairly intense so you will dilute with other oils to reduce the pungent property of the oil. Therefore, the study includes a combination between pumpkin oil with clove oil. The zones of inhibition decreased into 16 mm this may be the combination lead to antagonism while when combine with coconut oil lead to synergic effect (Table 8). Our results are compatible with Hicham *et al.* [51] who concluded that antifungal activity increased by the combination between clove oil and coconut oil. The lipophilic effect of the monoterpenes that found in essential oils give them the antimicrobial activity by disrupting microbial cytoplasmic membrane, then loss of impermeability for bigger ions and protons; it's function are compromised in barrier as well as the matrix of enzymes, and as an energy transducer if disturbance of membrane integrity occurs [50].

The combination result between plant oils and vinegar (Table 9) showed that vinegar doesn't increase the activity of clove oil at a same time appeared antagonism effect by decrease zone of inhibition when used vinegar with (clove oil with coconut oil) from 50mm into 22mm and (thyme oil) from 35 into 14mm. Individual components

each of clove oil, onion juice, thyme oil, hydrogen peroxide, Sodium chloride, lugol's

iodine, Detol, apple cider vinegar confirmed high activity against *C. albicans*.

Table 7: Effects of some plant oils on growth of *C. albicans*.

Types of plant oils	Coconut oil	Castor oil	Sweet almond oil	Clove oil	Onion juice 50%	Garlic juice 50%	Olive oil	Pumpkin oil	Rosemary oil	Thyme oil	Tannins Oil	Pine oil
Zone of inhibition in millimeter	Re	Re	Re	45	45	Re	Re	Re	9	35	Re	Re

*Re= resistant (no inhibition zone)

Table 8: The combination effects are between plant oil on growth of *C. albicans*.

Types of plant oils	Clove oil+ Coconut oil	Pumpkin oil+ Clove oil	Pine oil+ Tannins oil	Thyme oil+ Pine oil	Rosemary oil+ Tannins oil	Olive oil+ Pine oil	Rosemary oil+ Thyme oil	Olive oil+ Tannins oil	Thyme oil+ Coconut oil
Zone of inhibition in millimeter	50	16	Re	Re	Re	Re	Re	Re	Re

*Re= resistant (no inhibition zone)

Table 9: Combinations between plant oils and apple cider vinegar.

Zone of inhibition in millimeter	Combination between plant oils + 40% apple cider vinegar (v/v)	Zone of inhibition in millimeter	Types of plant oils + 20% apple cider vinegar (v/v)	Zone of inhibition in millimeter
22	Clove oil+ Coconut oil	45	Clove oil	22
Re	Thyme oil+ Pine oil	14	Thyme oil	Re
Re	Rosemary oil+ Tannins oil	Re	Tannins Oil	Re
Re	Pine oil+ Tannins oil	Re	Coconut oil	Re
Re	Olive oil+ Pine oil	Re	Rosemary oil	Re
Re	Rosemary oil +Thyme oil	Re	Olive oil	Re
Re	Olive oil+ Tannins oil	Re	Pine nut oil	Re
Re	Thyme oil+ Coconut oil	Re	Pumpkin oil	Re

Re= resistant (no inhibition zone)

Conclusion

Solitary use of compounds (clove oil, onion juice, thyme oil, H₂O₂, NaCl, lugol's iodine, detol, and apple cider vinegar) was associated with high antifungal response regarding *C. albicans*; efficacy was reduced when used in combination. In exception to above finding synergistic effect was identified when a combination between clove oil and coconut oil.

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