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Evaluation of in Vitro Antifungal Activity of Garlic, Thyme, Aloe Vera, and Cinnamon Essential Oils against Candida Albicans, Aspergillus Flavus, Aspergillus Niger and Mucor Himalis

Mohammad Hossein Sadeghi Zali [1], Saber Yousefi [2], Amin Hashempour[3], Nazereh Hashempour[3], Saman Seyedgholizadeh [*3]

1. Department of Bacteriology, Urmia Branch, Islamic Azad University, Urmia, Iran
2. Department of Microbiology, Virology and Immunology, Urmia University of Medical Sciences, Urmia, Iran
3. Young Researcher and Elites Club, Urmia Branch, Islamic Azad University, Urmia, Iran

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***Corresponding author:**
Saman Seyedgholizadeh. Young Researcher and Elites Club, Urmia Branch, Islamic Azad University, Urmia, Iran
Phone: +989120897092
Fax: +982188384877
Email:saman.seyedgholizade@gmail.com
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Abstract

Introduction: Antifungal drugs have major limitations such as fungal resistance to classical drugs and treatment costs. It has been reported that antifungal activity of some essential oils as safe combinations. Thus, the present study was conducted to investigate the antifungal activity of garlic, thyme, aloe vera and cinnamon essential oils against Candida albicans, Aspergillus flavus, Aspergillus niger and Mucor himalis.

Methods: Antibacterial activities of essential oils were evaluated by disk diffusion assay. The data were collected for 1, 3, 7 and 10 days.

Results: Findings indicated that all essential oils showed similar antifungal activity against A. flavus, A. niger and M. himalis. Garlic essential oil was more efficient against C. albicans compared with other essential oils.

Conclusion: The selected essential oils can efficiently show antifungal activity against A. flavus, A. niger and M. himalis, but garlic essential oil showed antifungal activity against the selected fungus Thus, garlic essential oil can be suggested as helper for common antifungal drugs.

Keywords: Antifungal Activity, Candida Albicans, Disk Diffusion Assay, Essential Oils, Garlic

Introduction

Today, fungi are known as the most important pathogens. In the past years, fungal infections created by opportunistic microorganisms have been caused major concerns for clinical importance (1-3). On the basis previous studies, the prevalence of opportunistic infections, especially in hospital environments, is allocated to the genera Candida and Aspergillus (4). It is well accepted that Candida is known as the third or fourth common infection in the USA. It has been shown candidosis is the most common invasive fungal infection in patients with non-neutropenia disease (5). Dermatomycoses, especially dermatophytes, have been known as the most common infections caused by members of the genus Candida. Superficial candidosis and dermatophytosis can be severe in patients with immune deficiency. The aspergilli are highly distributed in the human environment. Aspergillus flavus is the name now applied to describe a species as well as a group of closely related species (6). The presence of Aspergillus in the air is known as a major risk factor for both invasive and allergic aspergillosis (7). Several molecular

studies have shown the relation between A. flavus and the contamination of the environment (8, 9). There was increasing mortality due to invasive aspergillosis during 1980 to 1997 years (10). Aspergillus niger is known as a factor for mould production which is rarely shown as responsible for pneumonia. A. niger has been related with otomycosis (11), cutaneous infections (12) and pulmonary disease. There has been a significant increase in infections due to emerging fungi, such as Mucor (1, 2).

However, antifungal drugs have major limitations such as fungal resistance to classical drugs and the treatment costs (13). These limitations enforce to use the alternatives for antifungals and/or use of combination treatments for fungal infections. Today, aromatic plants have been widely applied in folk medicine. This activity of aromatic plants may be attributed to their volatile oils. The antifungal activity of some essential oils have been reported (14). Previous studies have shown antifungal activity of essential oils against yeasts, dermatophyte fungi and Aspergillus strains (15-17). Allium sativum (garlic) is member of Alliaceae family. It has been used as bactericide (18), anti-trypanosom (19), etc… Thymus vulgaris (Thyme) is a medicinal plant belonging to Lamiaceae family which is highly found worlwide worldwide and used for culinary, cosmetic perennial and medical purposes. It has been accepted it acts as as antispasmodic, expectorant, antiseptic, antimicrobial and antioxidant (20). Aloe vera is member of Lilaceae family and its anti-inflammatory activity (21), immuno stimulatory activity (22) and cell growth stimulatory activity (23). Have been accepted. Cinnamomum zylenicum (cinnamon) is widely applied in food industry because of its special aroma. It has been widely used as strong antibacterial, anticandidial, antiulcer, analgesic and antioxidant (24). So far no study has been investigated to compare antifungal activity of garlic, thyme, aloe vera and cinnamon essential oils against Candida albicans, Aspergillus flavus, Aspergillus niger and Mucor himalis. Thus the present study was conducted to investigate and compare in vitro antifungal activity of garlic, thyme, aloe vera and cinnamon against C.albicans, A.flavus, A.niger and M.himalis.

Methods

Plant essential oils of garlic, thyme, aloe vera and cinnamon were prepared. The studied essential oils were isolated by the hydro-distillation method by a clevenger-type apparatus as previously explained by Lamaty et al, 1987 (25). The recovered oils were dried over anhydrous sodium sulfate and stored in darkness between 4 and 6°C.

All fungal strains were prepared from Scientific and Industrial Research Center of Iran. The strains were then sub-cultured in subord dextrose agar culture medium. The PTCC numbers for funguses were as follows; C. albicans (5027), A.flavus (5006), A.niger (5010) and Mucor nidicola (5292). For disk diffusion assay, Antibacterial activities of essential oils were evaluated as described previously by Bauer et al, 1996 (26). Summary, culture mediums were sterilized at 121°C for 20 minutes. Firstly, fungi were cultured, in punch form, on subord dextrose agar culture medium. Punch form is defined as pick up part of culture medium and replacement of the same size fungal strain instead of it. Then, essential oil-included discs were placed on punch position (concentration of essential oils was 100% and sterilized disks were positioned into essential oil for 15 minutes). They were subsequently incubated for 10 days. They were investigated for diameter of inhibition zone during 1, 3, 7 and 10 days. For data analysis, The experiment was conducted in a completely randomized design with 4 replicates for each essential oil. data were analyzed by using SPSS software (version 22). The data are presented as mean± standard deviation (SD).

Results

Antifungal activity of essential oils against the selected fungi is presented in Table 1. As results indicate the studied essential oils similarly showed antifungal activity against A.flavus, A.niger and M.himalis ($P<0.05$). Considering mean± SD, garlic essential oil could show antifungal activity against C. albicans compared with other essential oils at day 1 [8.00±00 for garlic essential oil, 5.625±0.75 for thyme essential oil, 2.75±0.288 for aloe vera essential oil and 5.469±0.707 for essential oil]. As time increases (3, 7 and 10 days) differences between thyme and garlic essential oil reduce. In conclusion, it can be stated that garlic essential oil can efficiently show antifungal activity against C. albicans compared with other essential oils.

Table 1. Antifungal activity of essential oils against the selected fungus (diameter of inhibition zone in cm) at days of 1, 3, 7 and 10

Parameters	Garlic	Thyme	Aloe vera	Cinnamon
C.albicans1	8.00±00	5.625±0.75	2.75±0.288	5.469±0.707
C.albicans3	5.625±0.75	5.625±0.75	2.50±0.288	4.875±0.707
C.albicans7	5.625±0.25	4.875±0.25	2.50±0.480	4.375±0.408
C.albicans10	4.875±0.50	4.25±0.50	2.25±0.288	3.969±0.408
A.flavus1	8.00±00	8.00±00	8.00±00	8.00±00
A.flavus3	8.00±00	8.00±00	8.00±00	8.00±00
A.flavus7	8.00±00	8.00±00	7.00±00	7.75±00
A.flavus10	8.00±00	8.00±00	7.00±00	7.75±00
A.niger1	8.00±00	8.00±00	8.00±00	8.00±00
A.niger3	8.00±00	8.00±00	7.00±00	7.75±00
A.niger7	8.00±00	8.00±00	7.00±00	7.75±00
A.niger10	8.00±00	8.00±00	7.00±00	7.75±00
M.himalis1	8.00±00	8.00±00	8.00±00	8.00±00
M.himalis3	8.00±00	8.00±00	8.00±00	8.00±00
M.himalis7	8.00±00	8.00±00	8.00±00	8.00±00
M.himalis10	8.00±00	8.00±00	8.00±00	8.00±00
Control1	8.00±00	8.00±00	8.00±00	8.00±00
Control3	2.50±00	2.50±00	2.50±00	2.50±00
Control7	0.00±00	0.00±00	0.00±00	0.00±00
Control10	0.00±00	0.00±00	0.00±00	0.00±00

Discussion

Our findings indicate that essential oils showed antifungal activity against A. flavus, A. niger and M. himalis. Although they showed antifungal activity against C. albicans, garlic essential oil could efficiently show antifungal activity against C. albicans. It is well known that fungi are essential part of biological cycle which can create several damages to the environment, building materials and the health of human and animals, if the fungal concentration to be elevated. Antifungal activity of some essential oils has been previously reported (27-30). Particularly, antifungal activity of Thymus and their phenolic components have been reported (15, 31-33). Cock (2008) also examined that Aloe vera gel has inhibitory effect on A. niger. All the mentioned studies well show antifungal activity of plant derivatives (34). It is generally accepted that essential oil

components show their activity against the structure of the cell membrane (35). A study has shown that low concentrations of essential oils can change cell structure, inhibit respiration and change permeability of the cell membrane, while higher concentrations can cause membrane damage, loss of homeostasis and cell death (36). Antifungal activity of essential oils may be attributed to interaction between enzymes responsible for energy production and the synthesis of structural compounds of the cell with essential oil components (37). A study has suggested that essential oil components can pass by cell membrane and interact with its protein and finally create changes leading to death (38).There are other mechanism for antifungal activity of essential oils. Other researchers suggested that hydrogen bonds formation between hydroxyl groups of phenolic compounds and active sites of cellular enzymes may be responsible for antifungal activity of essential oils (39). Sharma and Tripathi (2006) reported that the active components may cause loss of integrity of the cell wall, and subsequently cause death (40). All the mentioned studies well state antifungal activity of essential oils. In the present study, no mechanism was investigated and we cannot clearly state that these essential oils act through that mechanism. These essential oils may act by one mechanism and/or more. Interestingly, garlic essential oil could show antifungal activity against C. albicans. This activity may be attributed to sulfuric-included components. Garlic essential oil components have been shown to have inhibiting activity of the enzymes (41). This activity can subsequently influence the synthesis of fatty acids, lipids, DNA or RNA.

Conclusion

Results indicated that garlic, thyme, cinnamon and aloe vera showed antifungal activity against A. flavus, A. niger and M. himalis. However, garlic essential oil could show antifungal activity against C. albicans. Antifungal activity may be attributed to essential oil components. Garlic essential oil was more efficient compared with other essential oils, thus it can be suggested as a helper in combination with other common antifungal drugs.

Ethical Issues

No applicable.

Authors' Contributions

Saman seyed gholizadeh is corresponding author authors' contributions is 35 % and other author is 16.25 %.

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References

1. Kauffman CA. Fungal infections. Proc Am Thorac Soc. 2006; 3: 35- 40.
2. Enoch DA, Ludlam HA, Brown NM. Invasive fungal infections: A review of epidemiology and management options. J Med Microbiol. 2006; 55: 809- 818.
3. Cruz MCS, Santos PO, Barbosa AM JR, Mélo DLFM, Alviano CS, Antoniolli AR, Alviano DS, Trindade RC. Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. J Ethnopharmacol. 2007; 111: 409- 412.
4. Singh N. Impact of current transplantation practices on the changing epidemiology of infections in transplant recipients. Lancet Infect Dis. 2003; 3: 156- 161.
5. Eggimann P, Garbino J, Pittet D. Management of candida species infections

in critically ill patients. Lancet Infect Dis. 2003; 3: 772- 785.
6. Link HF. Observationes in ordines plantarum naturales. Dissertatio prima complectens Anandrarum ordines Epiphytas, Mucedines, Gastromycos et Fungos. Gesellschaft fur Natur (Berlin). 1809; 3: 3- 42.
7. Denning DW. Invasive aspergillosis. Clin Infect Dis. 1998; 26: 781- 803.
8. Rath PM, Ansorg R. Value of environmental sampling and molecular typing of aspergilli to assess nosocomial sources of aspergillosis. J Hosp Infect. 1997; 37: 47- 53.
9. Diaz-Guerra TM, Mellado E, Cuenca-Estrella M, Gaztelurrutia L, Navarro JIV, Tudela JLR. Genetic similarity among one Aspergillus flavus strain isolated from a patient who underwent heart surgery and two environmental strains obtained from the operating room. J Clin Microbiol. 2000; 38: 2419- 2422.
10. McNeil MM, Nash SL, Hajjeh RA, Phelan MA, Conn LA, Plikaytis BP, Warnock DW. Trends in mortality due to invasive mycotic diseases in the United States, 1980-1997. Clin Infect Dis. 2001; 33: 641-647.
11. Araiza J, Canseco P, Bonifaz A. Otomycosis: clinical and mycological study of 97 cases. Rev Laryngol Otol Rhinol (Bord). 2006; 127: 251- 254.
12. Loudon KW, Coke AP, Burnie JP, Shaw AJ, Oppenheim BA, Morris CQ. Kitchens as a source of Aspergillus niger infection. J Hosp Infect. 1996; 32: 191- 198.
13. Rapp RP. Changing strategies for the management of invasive fungal infections. Pharmacotherapy. 2004; 24: 4S- 28S.
14. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem. 2003; 10: 813- 829.
15. Pina-Vaz C, Rodrigues AG, Pinto E, Costa-de-Oliveira S, Tavares C, Salgueiro LR, Cavaleiro C, Gonc alves MJ, Martinez-de-Oliveira J. Antifungal activity of Thymus oils and their major compounds. J Eur Acad Dermatol. 2004; 18: 73- 78.
16. Salgueiro LR, Cavaleiro C, Pinto E, et al. Chemical composition and antifungal activity of the essential oil of Origanum virens on Candida species. Planta Med. 2003; 69: 871- 874.
17. Salgueiro LR, Pinto E, Gonc alves MJ, et al. Chemical composition and antifungal activity of the essential oil of Thymbra capitata. Planta Med. 2004; 70: 572- 575.
18. Blackwood J, Fulder S. Garlic; nature's original remedy. Javelin Books. 1986.
19. Yusuf KO, Ekanem JT. Studies of phytochemical constituents and antitrypanosomal properties of fermented wheat germ and garlic bulbs extract on Trypanosoma brucei- infected rats. J Med Plants Res. 2010; 4 (19): 2016- 2020.
20. Abu-Darwish MS, Abu-Dieyeh ZH, Batarseh M, Al-Tawaha ARM, Al- Dalian SYA. Trace element contents and essential oil yields from wild thyme plant (Thymus serpyllum L) growth at different natural variable environments. Jordan J Food Agric Environ. 2009; 7: 920- 924.
21. Malterud KE, Fabrot TL, Huse AE. Antioxidant and radical scavenging effects of anthraquinones and anthrones. Pharmacology. 1993; 47 (1): 77- 85.
22. Ramamoorthy L, Tizard IR. Induction of opoptosis in a macrophage cell line RAW 264.7. Molecular Pharm. 1998; 53: 415-421.
23. Tizard I, Busbee D, Maxwell B, Mc K. Effect of acemannan, a complex carbohydrate, on wound healing in young and aged rats. Wounds. 1994; 6: 201- 209.
24. Ciftci M, Simsek UG, Yuce A, Yilmaz O, Dalkilic B. Effects of dietary antibiotic and cinnamon oil supplementation on antioxidant enzyme activities, cholesterol levels and fatty acid compositions of

serum and meat in broiler chickens. Acta Vet Brno. 2010; 79: 33- 40.
25. Lamaty G, Menut C, Bessiere JM, Zollo PHA, Fekam F. Aromatic plants of tropical Central Africa. I. Volatile components of two annonaceae from Cameroon: Xylopia aethiopica (Dunal) A. Richard and Monodora myristica (Gaerth.) Dunal Flavours Fragrance J. 1987; 2: 91- 94.
26. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc method. Am J Clin Pathol. 1996; 44: 493- 496.
27. Baydar H, Sagdic O, Ozkan G, Karadogan T. Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey. Food Control. 2004; 15: 169- 172.
28. Nostro A, Blanco A R, Cannatelli M A, Enea V, Flamini G, Morelli I, Roccaro A S, Alonzo V. Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. FEMS Microbiol Lett. 2004; 230: 191- 195.
29. Valero M, Salmeron MC. Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. Int J Food Microbiol. 2003; 85: 73- 81.
30. Dewang PM, Nikumbh VD, Tare VS, Mahulikar PP. Eco-friendly pest management using monoterpenoids II Antifungal efficacy of Menthol Derivatives. J Sci Ind Res. 2003; 62: 990- 995.
31. Rasooli I, Abyaneh M R. Inhibitory effects of Thyme oils on growth and aflatoxin production by Aspergillus parasiticus. Food Control. 2004; 15: 479- 483.
32. Tullio V, Nostro A, Mandras N, Dugo P, Banche G, Cannatelli M A, Cuffini A M. Alonzo V, Carlone NA. Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. J Appl Microbiol. 2007; 102: 1544- 1550.
33. Klaric MS, Kosalec I, Mastelic J, Pieckova E, Pepeljnak S. Antifungal activity of thyme (Thymus vulgaris L.) essential oil and thymol against moulds from damp dwellings. Lett Appl Microbiol. 2007; 44: 36- 42.
34. Cock IE. Antimicrobial activity of Aloe barbadensis Miller leaf gel components. Int J Microbiology. 2008; 4 (2): 1937- 8289.
35. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez J. Antifungal activity of lemon (Citrus lemon L.), mandarin (Citrus reticulata L.), grapefruit (Citrus paradisi L.) and orange (Citrus sinensis L.) essential oils. Food Control. 2008; 19: 1130- 1138.
36. Carson CF, Mee BJ, Riley TV. Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrobial Agents and Chemotherapy. 2002; 46: 1914- 1920.
37. Conner DE, Beuchat LR. Effect of essential oils from plants on growth of food spoilage yeasts. J Food Sci. 1984; 49: 429- 434.
38. Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H. Antifungal activity of thyme, summer savory and clove essential oils against Aspergillus flavus in liquid medium and tomato paste. Food Control. 2007; 18: 1518- 23.
39. Daferera DJ, Ziogas BN, Polissiou MG. GC–MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicillium digitatum. J Agricultural Food Chem. 2000; 48: 2576- 2581.
40. Sharma N, Tripathi A. Effects of citrus sinensis (L.) Osbeck epecarp essential oil on growth and morphogenesis of

Aspergillus niger (L.) Van Tieghem. Microbiological Res. 2006; 163: 337- 344.

41. Beuchat LR, Golden DA. Antimicrobials occurring naturally in foods. Food Tech. 1989; 43: 134- 142.