Environmental Health Engineering and Management Journal 2015, 2(1), 13–16

http ehemj.com

Environmental Health Engineering and Management Journal

Original Article

Environmental Health Engineering and Management

Open Access Publish Free

Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant

Mohammad Taghi Ghaneian[1], Mohammad Hassan Ehrampoush[2], Ali Jebali[3], Seyedhossein Hekmatimoghaddam[3], Mohammad Mahmoudi[4*]

[1]Associate Professor, Department of Environmental Health, Faculty of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

[2]Professor, Department of Environmental Health, Faculty of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

[3]Assistant Professor, Department of Laboratory Sciences, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

[4]MSc Student, Department of Environmental Health, Faculty of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Abstract

Background: Although various surface disinfectants have been introduced, most of them are toxic. The use of natural antimicrobial agent e.g. phytol, extracted from *Leptadenia pyrotechnica* is a new strategy. The aim of this study was to evaluate the antimicrobial activity, toxicity, and stability of phytol.

Methods: The serial concentrations of phytol were prepared, and separately incubated with four microbial isolates. Then, its Minimum Inhibitory Concentration (MIC) was measured for each microorganism. For toxicity test, serial concentrations (62.5, 125, 250, 500 and 1000 µg/mL) of phytol were incubated with mouse skin cells, and then cell viability was calculated by MTT assay. For stability test, three common surfaces (stone, steel, and MDF) were considered. Then, 100 µL of phytol was separately spread over their surface, and they have been kept at lab panel for 12, 24 and 36 hours. After incubation, two samples were obtained from each surface and inoculated on nutrient agar plates. Finally, colony count was read for each surface. T-test was used to evaluate the significant differences between groups, and P>0.05 considered as level of significant difference.

Results: The MIC50 of phytol against *E.coli, C.albicans*, and *A.niger* was 62.5 µg/mL, and against *S.aureus* was >1000 µg/mL. MTT assay showed that the toxicity of phytol was dose and time dependent. The stability test demonstrated that phytol was stable on the stone, MDF, and steel surfaces until 36 hours.

Conclusion: It can be concluded that phytol has high antimicrobial activity, high stability, and low toxicity. This substance must be evaluated at actual conditions.

Keywords: Antimicrobial activity, Phytol, Toxicity, Stability

Citation: Ghaneian MT, Ehrampoush MH, Jebali A, Hekmati Moghaddam SH, Mahmoudi M. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environmental Health Engineering and Management Journal 2015; 2(1): 13–16.

Article History:
Received: 18 September 2014
Accepted: 7 January 2015
ePublished: 20 January 2015

***Correspondence to:**
Mohammad Mahmoudi
Email: mahmoodi170@yahoo.com

Introduction

Elimination of pathogenic microorganisms such as bacteria, molds, yeasts and viruses is one of the main concerns of human. Historically, so many natural and synthetic materials have been used for this purpose. Also, various disinfectants including Deconex, Microten, Cidex, and Alprocide have been introduced to remove pathogens from surfaces. Isopropyl alcohol and ethanol were applied to reduce pollution and to minimize nosocomial infections in medical centers. Moreover, to eliminate pathogenic fungi on work surfaces, different materials such as Betadine, Detol, and Savlon have been applied (1-3).

Following the failure of antibiotics and the emergence of new resistant strains, nowadays, scientists are interested to work on plant antimicrobial agents. Several researchers have studied the antimicrobial effects of essential oils, and it has been established that certain essential oils have antiseptic and antimicrobial effects (4,5).

Leptadenia pyrotechnica is a desert plant, and grows in tropical regions of Pakistan, India, Iran, Saudi Arabia, Egypt, Sudan, Somalia, Chad, Libya, and Algeria. The plant has been used as an anti-seizure, anti-inflammatory, anti-allergic, and anti-bacterial material. Also, new studies have proven that this plant is analgesic, anabolic, astringent, and laxative (6-8). Studies have shown that *L. Pyrotechnica* has some bioactive compounds such as phytols, steroidal glycosides, cardenoids, alkaloids, flavonoids, and terpenes. Phytol (3,7,11,15-tetramethylhexadec-2-EN-1-OL) is an important member of branched chain unsaturated terpene, and is a product of chlorophyll metabolism in plants. Interestingly, phytol can inhibit microbes (6-8).

The aim of this study was: 1) to evaluate the antimicrobial activity of phytol, 2) to evaluate its toxicity on mouse skin cells, and 3) to evaluate its stability on different surfaces.

Methods

Preparation of microbial strains

The standard strain of *Escherichia coli* and *Staphylococcus aureus* was obtained from Iranian Research Organization for Science and Technology (IROST). These strains were cultured on nutrient agar (Invitrogen, UK) at 37 °C (Table 1). Also, standard strain of *Candida albicans* and *Aspergillus niger* provided from IROST were cultured on Sabouraud dextrose agar (Invitrogen, UK) at 25 °C. For each strain, a single colony was added to Mueller Hinton broth (Invitrogen, UK) to reach ½ McFarland.

Antimicrobial test

At this stage, 100 µL serial concentrations (62.5, 125, 250, 500 and 1000 µg/mL) of phytol (Sigma-Aldrich, USA) were prepared in 96-well plates. In each well, 100 µL of fungal and bacterial suspension was separately added. Then, bacterial and fungal strains were incubated at 37 °C and 25 °C for 24 hours, respectively. After incubation, the optical density of each well was determined by ELISA reader (Novin Gostar, Iran) at 405 nm. In the control group, 100 µL of distilled water was separately added to bacterial and fungal strains, and incubated in the same way as test wells (9).

Toxicity test

To evaluate the toxicity of phytol,3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay was used. First, serial concentrations (62.5, 125, 250, 500, 1000 µg/mL) of phytol were prepared. Then, 100 µL of each concentration was added to 100 µL of mouse skin suspension, and incubated for 12, 24, and 36 hours at 37 °C. Mouse skin suspension was prepared according to standard protocol (10), and adjusted at 10,000 cells/mL. After incubation, 25 µL of 5- mg/mL MTT (Sigma-Aldrich, USA) was added to each well and then was incubated at 37 °C for 3 hours. Finally, the optical density of each well was read by ELISA reader at 492 nm. Such as antimicrobial test, in control well, 100 µL of distilled water was added to 100 µL of mouse skin suspension. Finally, the percentage of cell viability was calculated (10).

Stability test

In the first step, three surface models including stone, steel, and MDF were purchased from different shops of Yazd. All surfaces were washed by 70% ethanol. Then, 100 µL of phytol at 1000 µg/mL was separately added to all surfaces, spread using a sterile swab, and dried at room temperature. All surfaces were maintained at lab panel for 12, 24, and 36 hours at room temperature. After incubation, two samples were obtained from each surface using a sterile swab, and inoculated on nutrient agar plates under sterile conditions. Then, the cultures were incubated for 48 hours at 37 °C, and finally the number of colonies grown on each plate was counted (11).

Table 1. Standard microbial strains which used in this study

Species	ATCC
E.coli	25922
S.aureus	25923
C.albicans	10231
A.niger	16888

ATCC= American Type Culture Collection

Statistical analysis

All experiments were done three times, and the results were shown as mean ± standard deviation. Here, T-test was used to evaluate significant differences between groups at P>0.05.

Results

The MIC50 and MIC90 of phytol against two bacterial and fungal strains are shown in Table 2. As seen, MIC50 of phytol against *E.coli*, *C.albicans*, and *A.niger* was 62.5 µg/mL, and against *S.aureus* was >1000 µg/mL (P<0.05). It means that *S. aureus* is resistant to phytol. The MIC90 of phytol against all strains was more than 1000 µg/mL, i.e. none of concentrations could inhibit 90% of microbial strains.

The results of MTT assay were shown in Figure 1. The results revealed the toxicity of phytol was dose-dependent, i.e. the minimum concentration (31.2 µg/mL) caused maximum cell viability (97%) and vice versa. Such a

Table 2. The MIC50 and MIC90 of phytol against microbial strains.

	MIC_{50} (µg/mL)	MIC_{50} (µg/mL)
Aspergillus Niger	62.5	>1000
Candida albicans	62.5	>1000
Escherichia coli	62.5	>1000
Staphylococcus aureus	>1000	>1000

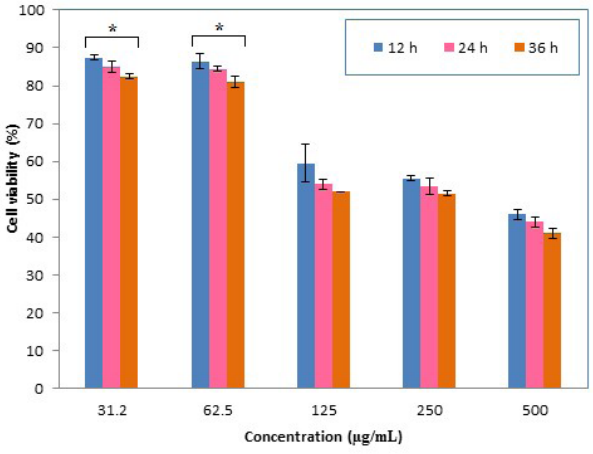

Figure 1. The results of MTT assay. *P<0.05 compared with 125, 250, and 500 µg/mL.

pattern was observed for all incubation times. Statistically, significant difference (P<0.05) was observed between the cell viability of phytol at concentration of 31.2 µg/mL and 62.5 µg/mL versus the cell viability of phytol at concentration of 125, 250, and 500 µg/mL. Another finding showed that the toxicity of phytol was time-dependent. Thus, the decrease of incubation time led to an increase of cell viability. Note that, the lowest cell viability (near 40%) which can be seen in this figure corresponds to concentration of 1000 µg/mL with incubation time of 36 hours (P<0.05) compared with 125, 250, and 500 µg/mL.

The results of stability test are shown in Table 3. As seen, the stone surfaces pre-treated with phytol and incubated for 12 and 24 hours at lab panel had the same colony count, but colony count was increased after 36 hours in both control and test surfaces. There were significant differences between colony count of pre-treated stone surfaces and that of control surfaces (P<0.05). Also, the MDF surfaces pre-treated with phytol had approximately the same colony count at three incubation times. Significant differences were also observed between the colony count of pre-treated MDF surfaces and that of control surfaces (P<0.05). As shown, the colony count of steel surfaces pre-treated with phytol and incubated for 12 hours was 2±2, but the colony count at other times was zero. Here, the significant differences were observed between pre-treated MDF surfaces and control (P<0.05). It can be revealed that phytol can maintain its stability on the stone, MDF, and steel surfaces over the 36-hour period.

Discussion

Although various disinfectants, e.g. Deconex, Microten, Cidex, and Alprocide have been introduced in the two last decades, most of them are toxic and unstable (1-3). In this study, we used phytol, extracted from *L. Pyrotechnica* as a new disinfectant. According to previous studies, this material is anti-inflammatory, anti-allergic, and anti-bacterial (6-8). Phytol (3,7,11,15-tetramethylhexadec-2-EN-1-OL) is an important member of the branched chain unsaturated terpene, and is a product of chlorophyll metabolism in the plant. The mechanism of its antimicrobial activity is not fully described. It is suggested that protein and enzyme inactivation is one of the important mechanisms for inactivation of microbes (6-8). In this study, antimicrobial activity of phytol, its toxicity on mouse skin cells, and its stability on different surfaces were investigated. We found that phytol is an approximately good antimicrobial agent. Moreover, it had no remarkable toxicity and had high stability.

Compared with other studies, the antimicrobial efficacy of phytol is comparable with other traditional disinfectants (12,13). In this section, some related articles have been reported to be compared with this study. Pejin *et al* considered on antiradical and antimicrobial activities of phytol. Its antiradical activity was evaluated by electron paramagnetic resonance. Also, its antimicrobial activity was evaluated by the microdilution method against eight bacterial and eight fungal strains. Its MIC was 0.003–0.038 mg/mL and 0.008–0.016 mg/mL against bacteria and fungi, respectively (14). Kumar *et al* studied the anthelminthic activity of *L. pyrotechnica.* Two concentrations (50 and 100 mg/ml) of methanolic extract were studied in this research. They declared that methanolic extract of this plant exhibited significant anthelmintic activity at highest concentration of 100 mg/ml (15).

Table 3. The results of stability test at 3 incubation times

	Control (cfu/cm²)	Phytol(cfu/cm²)
Stone		
12 h	63±5	2±2*
24 h	92±2	2±0*
36 h	183±3	7±7*
MDF		
12 h	65±2	1±1*
24 h	70±3	1±1
36 h	76±2	0*
Steel		
12 h	42±2	2±2*
24 h	105±2	0*
36 h	227±1	0*

*shows significant difference when compared with its control (P<0.05)

Conclusion

Based on the results, it can be concluded that phytol is a good choice for disinfection of surfaces, because: 1) it had antimicrobial activity against *E.coli*, *C.albicans*, and *A.niger* 2) it had no remarkable toxicity, and 3) it was stable on all three surfaces (stone, MDF, and steel) until 36 hours. This material must be evaluated at actual conditions in future studies.

Acknowledgments

The authors thank the laboratory staff of Yazd Pajoohesh Medical lab. This study was financially supported by Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Ethical issues

This study was carried out according to ethical committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. All animals were sacrificed under animal ethics, after experiments.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

In this study, MTG and MHE were supervisors of MM, as MSc student. Also, AJ and SHHM were advisors.

Reference

1. Rios MP, Morgano SM, Stein RS, Rose L. Effects of chemical disinfectant solutions on the stability and accuracy of the dental impression complex. J Prosthet Dent 1996; 76(4): 356-62.
2. Martin H. Chemical disinfectant and sterilant composition comprising acidic glutaraldehyde. Google Patents; 1984.
3. Mbithi JN, Springthorpe VS, Sattar SA. Chemical disinfection of hepatitis A virus on environmental surfaces. Appl Environ Microbiol 1990; 56(11): 3601-4.
4. Knobloch K, Pauli A, Iberl B, Weigand H, Weis N. Antibacterial and antifungal properties of essential oil components. Journal of Essential Oil Research 1989; 1(3): 119-28.
5. Lv F, Liang H, Yuan Q, Li C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Res Int 2011; 44(9): 3057-64.
6. Alqasoumi SI, Soliman G, Awaad AS, Donia A. Anti-inflammatory activity, safety and protective effects of Leptadenia pyrotechnica, Haloxylon salicornicum and Ochradenus baccatus in ulcerative colitis. Phytopharmacology 2012; 2(1): 58-71.
7. Khasawneh MA, Elwy HM, Hamza AA, Fawzi NM, Hassan AH. Antioxidant, anti-lipoxygenase and cytotoxic activity of Leptadenia pyrotechnica (Forssk.) decne polyphenolic constituents. Molecules 2011; 16(9): 7510-21.
8. Dagla HR, Paliwal A, Rathore MS, Shekhawat NS. Micropropagation of Leptadenia pyrotechnica (Forsk.) decne: a multipurpose plant of an arid environment. Journal of Sustainable Forestry 2012; 31(3): 283-93.
9. Pfaller MA, Castanheira M, Diekema DJ, Messer SA, Moet GJ, Jones RN. Comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Etest methods with the CLSI broth microdilution method for echinocandin susceptibility testing of Candida species. J Clin Microbiol 2010; 48(5): 1592-9.
10. Jebali A, Kazemi B. Triglyceride-coated nanoparticles: skin toxicity and effect of UV/IR irradiation on them. Toxicol in Vitro 2013; 27(6): 1847-54.
11. Chan LY, Zhang VM, Huang Yh, Waters NC, Bansal PS, Craik DJ, *et al*. Cyclization of the antimicrobial peptide gomesin with native chemical ligation: influences on stability and bioactivity. Chem Bio Chem 2013; 14(5): 617-24.
12. Munazir M, Qureshi R, Arshad M, Gulfraz M. Antibacterial activity of root and fruit extracts of Leptadenia pyrotechnica (asclepiadaceae) from Pakistan. Pak J Bot 2012; 44(4): 1209-13.
13. Khan ME, Hanatu I. Anti-nutritional/nutritional analysis and anti-microbial investigation of the ethanol extract of the stem bark of Leptadenia hastata (Asclepiadaceae). Journal of Environmental Treatment Techniques 2013; 1(3): 208-12.
14. Pejin B, Savic A, Sokovic M, Glamoclija J, Ciric A, Nikolic M, *et al*. Further in vitro evaluation of antiradical and antimicrobial activities of phytol. Nat Prod Res 2014; 28(6): 372-6.
15. Kumar S, Chaudhary S, Jha KK. Anthelminthic activity on the Leptadenia pyrotechnica (forsk.) decne. J Nat Prod Plant Resour 2011; 1(4): 56-9.