Original Research Article

Antifungal Activity of Phytochemicals against species of *Cladosporium* and *Cladophialophora*

ABSTRACT

Cladosporium species are ubiquitous, saprobic dematiaceous fungi, associated with human and animal opportunistic infections. *Cladosporium* has been known to be one of the most airborne fungi causing respiratory allergies diseases, particulary asthma and rhinitis. Antifungal compounds of natural origin, such as terpenes, have received much attention in recente times. They are a promising therapeutic tool for treating fungal infections, and are known for their antimicrobial properties. **Aims:** In this context, the present study aims to evaluate the in vitro antifungal activity of eight phytochemicals commonly found in *Melissa officinalis* L. essential oil (citral, (-) citronelal, (+) citronelal, β-caryophyllene, geraniol, linalool, β-cymene, α-pinene) against ten species of *Cladosporium* and *Chadophialophora*. **Methodology:** Antifungal susceptibility testing was performed with the phytochemicals at a concentration of 1.024 µg/mL. Microbiological screening was performed based on the broth microdilution technique. **Results:** Through analysis of results, it is observed that citral showed the best activities of the as species of *Cladosporium* studied. **Conclusion:** citral representing a new possibility in the arsenal of products for treatment of fungal infections caused by these fungi.

Keywords: **Keywords:** *Phytochemicals; Citral; Antifungal; Cladophialophora carrionii, Cladosporium oxysporum, Cladosporium sphaerospermum.*

1. INTRODUCTION

Fungal infections are becoming more frequent because of expansion of at-risk populations and use of treatment modalities that permit longer survival of these patients [1].

Cladosporium species are among the most common fungal inhabitants worldwide, being isolated from almost any environmental source and geographic location [2].

The most common *Cladosporium* species are primarily isolated from soil and plant material, where they are frequently encountered as saprobes or secondary invaders on follicular lesions, concomitant with other plant pathogenic fungi [2-4]. However, several species are important pathogens of plants and some are also able to affect animals including humans [5-7].

Cladosporium is usually associated with allergic rhinitis and asthma [8,9] or localized superficial or deep lesions [10-13], but rarely can cause disseminated infections [14,15]. They are difficult to treat due to long treatment periods, limited treatment options, resistance to common antifungal agents, and their greater prevalence among immunocompromised patients. All of these characteristics invite recurrences [16,17].

There exists a clear need for more, therapeutically effective antifungals. Actually, plants have been an interesting alternative to source of new biologically active compounds [18-20]. The plants produce numerous and varied organic compounds including monoterpenes and sesquiterpenes compounds present in essential oils, of which the majority does not directly participate in the plant's growth and development and are generally called secondary metabolites [21,22]

Melissa officinalis L., member of Lamiaceae family, is one of the well known aromatic medicinal plant species. The essential oil is a well-known antibacterial and antifungal agent [23-25]. There have

been some previous reports on the chemical constitutions of *M. officinalis.* According to these studies, the major components of the essential oil of *M. officinalis* were citral (geranial and neral), and citronellal [25,26], (*B*)-caryophyllene [27], caryophyllene oxide [27-28], linalool [29], geraniol [30], thymol [31], α-pinene [27], β-pinene [28,32], carvacrol and *iso*-menthone [33].

Previous studies in our laboratory with *M. officinalis* L. essential oil showed strong antifungal activity of this oil against *C. carrionii* strains (Menezes *et al*., 2015). Therefore, the aim of the present work was to investigate the antifungal activity of eight phytochemicals commonly found in *M. officinalis* L. essential oil against strains *of Cladosporium.*

2. MATERIAL AND METHODS

2.1 Phytochemicals and Synthetic Antifungal

The phytochemicals (citral, (-) citronelal, (+) citronelal, β-caryophyllene, geraniol, linalool, β-cymene, α-pinene) and amphotericin B (standard drug) were acquired from Sigma-Aldrich®. All them were dissolved in 2% Tween 80 (INLAB®) and up to 0.5 % dimethyl sulfoxide – DMSO (MERCK®) in sterile distilled water to obtain 2.048 µg/mL solutions.

2.2 Cladosporium Samples

For testing of antifungal activity were selected and used ten species of *Cladosporium. Cladophialophora carrionii* strains (URM 2871, 0212, CQ 02), *Cladosporium oxysporum* strains (URM 5234, URM 6056, URM 5412) and *Cladosporium spherospermum* strains (URM 5962, URM 5455, URM 5350, URM 6120) were taken from the Microorganisms Collection of the Mycology Laboratory, at the Department of Pharmaceutical Sciences, Health Sciences Center, Federal University of Paraíba, Brazil and from the Pernambuco (Brazil) Federal University, Biological Sciences Center – Mycology Department fungal collection (URM).

The fungal cultures were maintained on Sabouraud Dextrose Agar - SDA (DIFCO®) at room temperature (28° to 30℃) and under refrigeration (4℃).

2.3 Inoculum

Stock inoculate suspensions of the *Cladosporium* strains were prepared from 10-days old sabouraud dextrose agar (Difco Lab., USA) cultures grown at 28 ºC. Fungal colonies were covered with 5 mL of sterile saline solution (0.9 %), the surface was gently scraped with a sterile loop, and the saline solution with the fungal elements was transferred to a sterile tube. These suspensions were shaken for 2 min using a vortexer, and allowed to stand for 5 min to allow hyphal fragments to fall out of the suspensions so that the supernatant containing the conidia could be collected. Tubes containing the inocula were standardized to 0.5 McFarland scale (1-5 x 10^6 CFU/mL). Mold conidia were counted using a hemocytometer. The inocula of the conidial suspensions were adjusted using sterile NaCl 0.9 % to contain approximately 10^6 CFU/mL [34,35, 36].

2.4 Antifungal susceptibility testing

Antifungal susceptibility testing was performed with the phytochemicals (citral, (-) citronelal, (+) citronelal, β-caryophyllene, geraniol, linalool, β-cymene, α-pinene) at a concentration of 1.024 µg/mL. Antifungal susceptibility testing was performed based on the broth microdilution technique [34,36]. Sterile 96-U-shaped-well microplates were used and each well of the plates contained 100 µL of Sabouraud dextrose broth - SDB (DIFCO®). Then, 100 µL of the phytochemicals and antifungal drugs (2.048 µg/mL) were individually added to each line of wells, so that each line of wells corresponded to a phytochemical tested. Finally, 10 µL of fungal inoculum of each strain of *Cladosporium* were added to wells, so that each column corresponded to a strain. The microplates were incubated at 28 ℃ being selected those phytochemicals who showed better inhibition profile visual growth of microorganisms after seven days incubation. The standard antifungal was amphotericin B (1.024 µg/mL). Negative control (Sabouraud dextrose broth without drugs) was performed to confirm the viability of the conidia. Sensitivity control for

Tween 80 and DMSO was also performed. The assays were performed in triplicates, and the geometric mean values were calculated.

3. RESULTS AND DISCUSSION

The results of the antifungal susceptibility testing of phytochemicals against *Cladosporium* strains are summarized in Table 1.

In 1.024 µg/mL, the concentration of phytochemicals used, it was found that citral showed better antifungal activity, inhibiting the growth of 90 % of the *Cladosporium* strains tested. Resistant strains with only the *C. sphaerospermum* URM 5962. The phytochemicals (+) citronellal, linalool and α-pinene were able to inhibit the growth of at most 3 strains. The (-) citronellal inhibited the growth of 2 strains (*C. carrionii* URM 2871 and *C. carrionii* CQ 02), the β-caryophyllene and geraniol, inhibited only strains *C. sphaerospermum* URM 5962 and *C. carrionii* URM 2871, respectively and β-cymene was not able to inhibit the growth of any of the strains tested, at this concentration.

The term phytochemical relates to chemical compounds, non-nutritive, which naturally occur in plants and exhibit biological activity [37]. Studies involving phytochemicals are of great importance, because they facilitate the utilization of individual components, instead of a mixture like in essential oils, giving more predictability and probably less collateral effects. Several studies point to the various activities of phytochemicals, which are: antimicrobial, antioxidant, anti-inflammatory, analgesic, cardioprotective, anti-hemorrhagic, hepatoprotective, antitussive, antitumor, immunostimulating, anticancer, antiviral, among other [38-44].

Citral (3,7-dimethyl-2-6-octadienal) is the name given to a mixture of two geometric isomers: (2E)-3,7-dimetilocta-2,6-dienal (geranial, *trans*-citral, citral A) and (2Z)- 3,7-dimetilocta-2,6-dienal (neral, *cis*-citral, citral B), which are acyclic α, β-unsaturated monoterpene aldehydes that occur naturally in many essential citrus fruit oils and in other herbs or spices [45].

The citral aroma is stronger and sweeter than that of lemon [46]. Geranial has a strong lemon odor while neral has a sweeter, yet less intense lemon odor. Due to its characteristic lemon aroma, citral has become a flavoring substance of great importance, a heavily used rawmaterial for the pharmaceutical, food, perfume, and cosmetics industries [47,48]. Also it has emerged as the active component of citrus essential oils against pathogens [49].

Citral was reported by presenting antifungal activity [50-52], antibacterial [53,48], anti- *Leshimania* [54] anti-*Trypanosoma cruzi* [55] and insecticide [56].

In the present study, citral showed activity against *Cladosporium* strains, confirming the results obtained in previous studies. Zheng *et al*. [57] demonstrated the antimicrobial activity of citral front of fungal strains of *Penicillium digitatum*. Such phytochemical has brought an action against strains of methicillin-resistant *Staphylococcus aureus*, *Penicillium italicum* and *Rhizopus atolonifer* [58]. In recent studies, citral showed in vitro antifungal potential against strains of *Candida albicans* [59].

Knowing that there are few studies on the activity of essential oils and their phytochemicals in dematiaceous fungi, particularly on fungi of the *Cladosporium* genus, and that caused them infections are increasingly common around the world, this work will enable a contribution to scientific research, in respect to the pharmacological research of new antifungal products derived from natural products against these fungi.

Table 1. Antifungal activity of phytochemicals against species of Cladosporium - microdilution technique.

Microorganisms	Phytochemicals (1.024 µg/mL)										
	citral	(-) citronellal	(+) citronellal	β-caryophyllene	geraniol	linalool	β-cymene	α-pinene	**Amphotericin B**	**Culture control**	**Medium control**
C. carrionii URM 2871	-	-	+	+	-	+	+	-	-	+	-
C. carrionii 0212	-	+	+	+	+	+	+	+	+	+	-
C. carrionii CQ 02	-	-	+	+	+	+	+	+	-	+	-
C. oxysporum URM 5234	-	+	+	+	+	+	+	+	-	+	-
C. oxysporum URM 6056	-	+	+	+	+	-	+	+	+	+	-
C. oxysporum URM 5412	-	+	+	+	+	-	+	+	-	+	-
C. sphaerospermum URM 5962	+	+	+	-	+	-	+	-	-	+	-
C. sphaerospermum URM 5455	-	+	-	+	+	+	+	+	-	+	-
C. sphaerospermum URM 5350	-	+	-	+	+	+	+	-	-	+	-
C. sphaerospermum URM 6120	-	+	-	+	+	+	+	+	+	+	-

(+): Microbial growth in culture medium (-): Absence of microbial growth

4. CONCLUSION

The results obtained in this study show the pharmacological potential of plant products, particularly, the antifungal potential of citral against *Cladosporium* and *Cladophialophora*. The citral could appear as promising compound to be inserted in pharmaceutical formulations applied to control the survival and dissemination of etiological agents of superficial or systemic opportunistic mycoses. Moreover, the results of this study show the necessity of accomplishment of researches addressed to the evaluation of antimicrobial properties of this phytochemical in different pathogenic microorganisms.

REFERENCES

1. Naggie S, Perfect JR. Molds: hyalohyphomycosis, phaeohyphomycosis, and zygomycosis. Clin Chest Med, 2009; 30: 337–353.
2. Bensch K, Braun U, Groenewald JZ, Crous PW. The genus *Cladosporium*. Stud Mycol, 2012; 72:1–401.
3. Ellis MB. 1971. Dematiaceous hyphomycetes. CMI, Kew.
4. Ellis MB. 1976. More dematiaceous hyphomycetes. CMI, Kew.
5. De Hoog GS, Guarro J, Gené J, Figueras MJ. 2011. Atlas of clinical fungi. CD-ROM version 3.1, CBS-KNAW fungal biodiversity centre, Utrecht.

6. Ma X, Gu Y, Liu X, Li D, Ling S, Hou J, Wang C, Cao S, Huang X, Wen X, Ruan J, Dong C, Li C, Tong Y. Phaeohyphomycotic dermatitis in a giant panda (*Ailuropoda melanoleuca*) caused by *Cladosporium cladosporioides*. Med Mycol Case Rep, 2013; 2: 119–121.
7. Mercier E, Peters IR, Billen F, Battaille G, Clercx C, Day MJ, Peeters D. Potential role of *Alternaria* and *Cladosporium* species in canine lymphoplasmacytic rhinitis. J Small Anim Pract, 2013; 54: 179–183.
8. Chen BY, Chao HJ, Wu CF, Honda Y, Guo YL. High ambient *Cladosporium* spores were associated with reduced lung function in schoolchildren in a longitudinal study. Sci Total Environ, 2014; 481: 370-376.
9. Vicendese D, Dharmage SC, Tang ML, Olenko A, Allen KJ, Abramson MJ, Erbas B. Bedroom air quality and vacuuming frequency are associated with repeat child asthma hospital admissions. J Asthma: Official Journal of the Association for the Care of Asthma, 2014; 1-17.
10. Sang H, Zheng XE, Zhou WQ, He W, Lv GX, Shen YN, Kong QT, Liu WD. A case of subcutaneous phaeohyphomycosis caused by *Cladosporium cladosporioides* and its treatment. Mycoses, 2012; 55: 195–197.
11. Gugnani HC, Ramesh V, Sood N, Guarro J, Moin-Ul-Haq, Paliwal-Joshi A, Singh B. Cutaneous phaeohyphomycosis caused by *Cladosporium oxysporum* and its treatment with potassium iodide. Med Mycol, 2006; 44: 285–288.
12. Martinez-Herrera EO, Arroyo-Camarena S, Tajada-Garcia DL, Porras-Lopez F, Arenas R. Onychomycosis due to opportunistic molds. An Bras Dermatol, 2015; 90 (3): 334–337.
13. Sosa EE, Cohen PR, Tschen JA. *Cladosporium* scalp infection. Skinmed, 2012; 10 (6): 393-394.
14. Chen CY, Lee KM, Chang TC, Lai CC, Chang K, Lin CY, Chen YH. Acute meningitis caused by Cladosporium sphaerospermum. Am J Med Sci, 2013; 346(6):523-525.
15. Lalueza A, López-Medrano F, Del Palacio A, Alhambra A, Alvarez E, Ramos A, Pérez A, Lizasoain M, Meije Y, García-Reyne A, Aguado JM. *Cladosporium macrocarpum* brain abscess after endoscopic ultrasound guided celiac plexus block. Endoscopy, 2011; 43:E9–E10.
16. Abliz P, Fukushima K, Takizawa K, Nishimura K. Identification of pathogenic dematiaceous fungi and related taxa based on large subunit ribosomal DNA D1/D2 domain sequence analysis. FEMS Immunol Med Microbiol, 2004: 40: 41-49.
17. Bakheshwain S, Khizzi El, Rasheed AMAI, Ajlan AAI, Parvez S. Isolation of Opportunistic Fungi from Dermatophytic Samples. Asian J Dermatol, 2011: 3 (1): 13-19.
18. Nakamura CV, Ishida K, Faccin LC, Filho BP, Cortez DA, Rozental S, De Souza W, Ueda-T N. In vitro activity of essential oil from *Ocimum gratissimum* L. against four *Candida* species. Res Microbiol, 2004; 155(7): 579–586.
19. Oliveira DR, Leitão GG, Santos SS, Bisso HR, Lopes D, Alviano CS, Alviano DS, Leitão SG. Ethnopharmacological study of two *Lippia* species from Oriximina. Brazil. J Ethnopharmacol, 2006; 108 (1): 103-108.
20. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complement Altern Med, 2006; 6 (39): 1-8.

21. Monsálvez M, Zapata N, Vargas M, Berti M, Bittner M, Hernández V. Antifungal effects of n-hexane extract and essential oil of *Drimys winteri* bark against Take-All disease. Ind Crop Prod, 2010; 31: 239-244.
22. Corato U, Maccioni O, Trupo M, Di Sanzo G. Use of essential oil of *Laurus nobilis* obtained by means of a supercritical carbon dioxide technique against post harvest spoilage fungi. Crop Protection, 2010; 29: 142-147.
23. Abdellatif F, Boudjella H, Ziyouni A, Hassani A. Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian *Melissa officinalis* L. EXCLI Journal, 2014; 13: 772-781.
24. Jalal Z, Atki YE, Lyoussi B, Abdellaoui A. Phytochemistry of the essential oil of *Melissa officinalis* L. growing wild in Morocco:Preventive approach against nosocomial infections. Asian Pac J Trop Biomed, 2015; 5(6): 458–461.
25. Menezes CP, Guerra FQS, Pinheiro LS, Trajano VN, Pereira FO, Lima EO. Investigation of *Melissa officinalis* L. Essential Oil for Antifungal Activity against *Cladosporium carrionii*. IJTDH, 2015; 8(2): 49-56.
26. Moradkhani H, Sargsyan E, Bibak H, Naseri B, Sadat-Hosseini M, Fayazi-Barjin A, Meftahizade H. *Melissa officinalis* L., a valuable medicine plant. J Med Plants Res, 2010; 4: 2753-2759.
27. Norouzi, M.; Soleimani T, Pasha Zanousi M. Essential oil component in leaf and flower of Lemon balm (*Melissa officinalis* L.). Res Pharmaceut Sci, 2012; 7 (5): S749.
28. Basta A, Tzakou O and Couladis M. Composition of the leaves essential oil of *Melissa officinalis* from Greece. Flavour Fragr J, 2005; 20: 642-644.
29. Patora J, Majda T, Gora J and Klimek B. Variability in the content and composition of essential oil from Lemon balm (*Melissa officinalis* L.) cultivated in Poland. Acta Pol Pharm Drug Res, 2003; 60: 395-400.
30. Hussain AI, Anwar F, Iqbal T and Bhatti IA. Antioxidant attributes of four *Lamiaceae* essential oils. Pak J Bot, 2011; 43: 1315-1321.
31. Cosge B, Ipek A, Gurbuz B. GC/MS analysis of herbage essential oil from Lemon balms (*Melissa officinalis* L.) grown in Turkey. J Appl Biol Sci, 2009; 3: 149-152.
32. Sari AO and Ceylan A. Yield characteristics and essential oil composition of Lemon balm (*Melissa officinalis* L.) grown in the Aegean region of Turkey. Turk J Agric For, 2002; 26: 217-224.
33. Martino LD, Feo VD, Nazzaro F. Chemical composition and *in vitro* antimicrobial and mutagenic activities of seven Lamiaceae essential oils. Molecules, 2009; 14: 4213-4230.
34. Santos DA, Hamdan JS. Evaluation of broth microdilution antifungal susceptibility testing conditions for *Trichophyton rubrum*. J Clin Microbiol, 2005; 43 (4): 1917-1920.
35. Hadacek F, Greger H. Testing of antifungical natural products: methodologies, comparability of results and assay choise. Phytochem Anal, 2000; 11(3): 137-147.
36. Cleeland R, Squires E. 1991. Evaluation of new antimicrobials *in vitro* and in experimental animal infection. In: Antibiotics in Laboratory Medicine. New York: Williams & Wilkins.
37. Anjorin TS, Salako EA, Makun HA. Control of Toxigenic Fungi and Mycotoxins with Phytochemicals: Potentials and Challenges Mycotoxin and Food Safety in Developing Countries. InTech. 2013. Accessed 5 July 2013. Available:http://www.intechopen.com/book s/mycotoxin-and-food-safety-indevelopingcountries/control-of-toxigenicfungi-and-mycotoxins-withphytochemicals-

potentialsand- challenges" title="Control of Toxigenic Fungi and Mycotoxins with Phytochemicals: Potentials and Challenges">Control of Toxigenic Fungi and Mycotoxins with Phytochemicals: Potentials and Challenges

38. Wang L, Chen J, Xie H. Phytochemical profiles and antioxidant activity of adlay varieties. J Agric Food Chem, 2013; 61 (21): 5103–5113.
39. Thapa D, Losa R, Zweifel B, Wallace RJ. Sensitivity of pathogenic and commensal bacteria from the human colon to essential oils. Microbiology, 2012; 158: 2870–2877.
40. Naithani R, Huma LC, Holland LE. Antiviral activity of phytochemicals: a comprehensive review. Mini Rev Med Chem, 2008; 8 (11): 1106-33.
41. Marei GI, Rasoul MA, Abdelgaleil AS. Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. Pest Biochem Physiol, 2012; 103:56–61.
42. Kumar JK, Prasad AG, Richard AS. In vitro Antioxidant activity and preliminary phytochemical analysis of medicinal Legumes. J Pharmaceut Res, 2012; 5(6): 3059-3062.
43. Araújo-Júnior JX, Oliveira MS, Aquino PG. 2012. A phytochemical and ethnopharmacological review of the Genus Erythrina. In: RAO, V. (Ed.). Phytochemicals - A Global Perspective of Their Role in Nutrition and Health. New York: InTech.
44. Riju A, Sithara K, Nair SS. In silico screening major spice phytochemicals for their novel biological activity and pharmacological fitness. J Bioequiv Availab, 2009; 1: 063-073.
45. El Fattah MA, El Zahwey AM, Haridy IM, El Deeb SA, Menof. Effect of drying on the physicochemical properties and chemposition of lemongrass oil. J Agric Res, 1992; 17 (3): 1211-1230.
46. Rikanati RD, Sitrit Y, Tadmor Y, Iijima Y, Bilenko N, Bar E, Carmona B, Fallik E, Dudai N, Simon JE, Pichersky E, Lewinsohn E. Enrichment of tomato flavor by diversion of the early plastidial terpenoid pathway. Nat Biotechnol, 2007; 25: 899 – 901.
47. Leung AY and Foster S. 1996. Encyclopedia of common natural ingredient used in food, drugs and cosmetics. New York: John Wiley & Sons.
48. Marques AM, Lima CHP, Alviano DS, Alviano CS, Esteves RL, Kaplan MAC. Traditional use, chemical composition and antimicrobial activity of *Pectis brevipedunculata* essential oil: a correlated lemongrass species in Brazil. Emir J Food Agric, 2013; 25: 798–808.
49. Molina EG, Dominguez-Perles R, Moreno DA, Garcia-Viguera C. Natural bioactive compounds of *Citrus limon* for food and health. J Pharm Biomed Anal, 2010; 51:327–345.
50. Garcia R, Alves ESS, Santos MP, Viegas A, Fernandes AAR, Santos RB, Ventura JA, Fernandes PMB. Antimicrobial activity and potential use of monoterpenes as tropical fruits preservatives. Braz J Microbiol, 2008; 39: 163-168.
51. Zhou H, Tao N and Jia L, Antifungal activity of citral, octanal and α-terpineol against *Geotrichumcitri-aurantii*. Food Control, 2014; 37: 277–283.
52. Tao N, OuYang Q and Jia L. Citral inhibitsmycelial growth of *Penicillium italicum* by a membrane damage mechanism. Food Control, 2014; 41:116–121.
53. Belletti N, Kamdem SS, Tabanelli G, Lanciotti R and Gardini F. Modeling of combined effects of citral, linalool and β-pinene used against *Saccharomyces cerevisiae* in citrus-based beverages subjected to a mild heat treatment. Int J Food Microbiol, 2010; 136 (3): 283–289.

54. Machado M, Pires P, Dinis AM, Santos-Rosa M, Alves V. Salgueiro L, Cavaleiro C, Sousa MC. Monoterpenic aldehydes as potential anti-Leishmania agents: activity of *Cymbopogon citratus* and citral on *L. infantum*, *L. tropica* and *L. major*. Exp Parasitol, 2012; 130 (3): 223-231.
55. Cardoso and Soares MJ. In vitro effects of citral on *Trypanosoma cruzi* metacyclogenesis. Mem Inst Oswaldo Cruz, 2010; 105 (8): 1026–1032.
56. Rice PJ, Coats JR. Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J Econ Entomol, 1994; 87: 1172–1179.
57. Zheng S, Jing G, Wang X, Ouyang Q, Jia L, Tao N. Citral exerts its antifungal activity against *Penicillium digitatum* by affecting the mitochondrial morphology and function. Food Chem, 2015; 178: 76-81.
58. Saddiq AA, Khayyat AS. Chemical and antimicrobial studies of monoterpene: Citral. Pest Biochem Physiol, 2010; 98: 89-93.
59. Leite MCA, Bezerra AB, Sousa JP, Guerra FQS, Lima EO. Evaluation of Antifungal Activity and Mechanism of Action of Citral against *Candida albicans.* Evid Based Complement Alternat Med, 2014; Article ID 378280, 9 pages.