

Fungicidal effect of antimicrobial agents against environmental fungi by broth microdilution method

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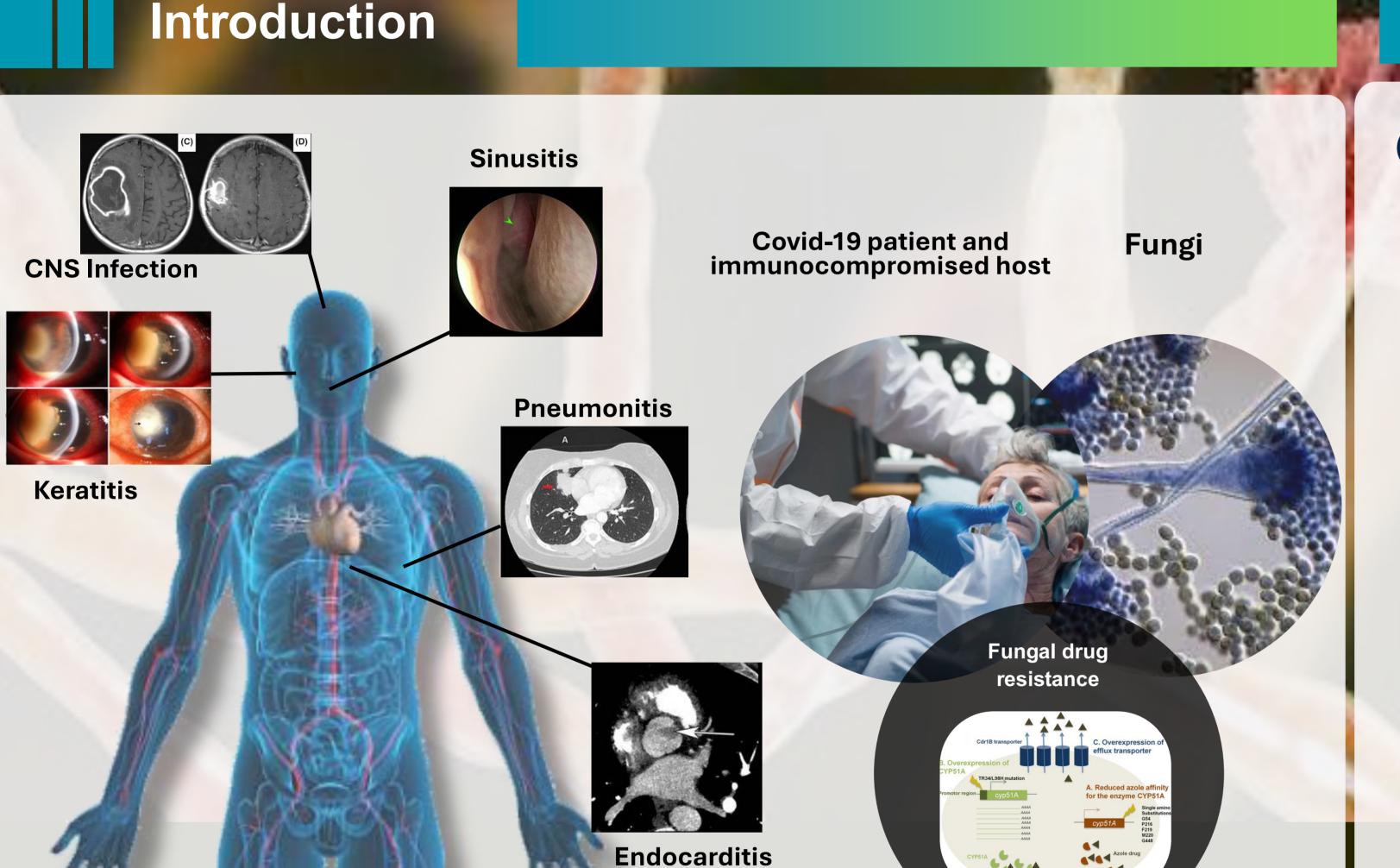
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Yordhathai Thongsri^{a*}, Namthip Amonnitichoksakul^b, Paweena Pamee^b, Suchaya Taraporn^b, Kanchana Usuwanthim^a, Pachuen Potup^a, and Sophit Khanthawong^c

- ^a Faculty of Allied Health Sciences Naresuan University Phitsanulok b Department of Medical Technology Faculty of Allied Health Sciences Naresuan University Phitsanulok
- ^c Department of Microbiology and Parasitology Faculty of Medical Sciences Naresuan University Phitsanulok *Corespondant author: E-mail address: yordhathait@nu.ac.th

ABSTRACT

Fungi cause infections in humans, animals, and plants particularly in immuno-compromised hosts that show a higher incidence of antimicrobial resistance. This study aims to evaluate the antifungal activity of six antimicrobial agents (ampicilin, rifampicin, chloramphenicol, amphotericin B, ketoconazole, and thimerosal) against five environmental fungal isolates (Aspergillus niger, Curvularia spp., Fusarium spp., Penicillium spp. and Rhizopus arrhizus) using the broth microdilution method. The results indicated that R. arrhizus resistance to ampicillin, chloramphenicol, and rifampicin with MIC values of > 1,600 and 50 µg/mL respectively. It was susceptible to amphotericin B, ketoconazole, chloramphenicol, and thimerosal at a concentration of ≤ 12.5 µg/mL. The four remaining fungal species could not determine. Further evaluation by disc diffusion method revealed that ampicillin could inhibited 20% of all fungal isolates, Curvularia spp. could inhibited at 100 µg/mL. Amphotericin B inhibited two fungal strains (40%), Curvularia spp. at 100 µg/mL and A. niger at the lowest concentration of ≤ 12.5 µg/mL. Ketoconazole could inhibit 60% of all fungal isolates, Curvularia spp. Fusarium spp. and Penicillium spp. could inhibited at 100, 400, and 800 µg/mL respectively. Thimerosal could inhibit all five fungal species (MIC ≤ 12.5 to 100 µg/mL). All fungal isolates exhibited 100% resistance to chloramphenicol and rifampicin. This research provides the inhibitory effect of antifungal agents against environmental fungi, which could serve as a guideline for treatment strategies of fungal infections in patients in the future.



Aim of the study

IE – inhibitory effect

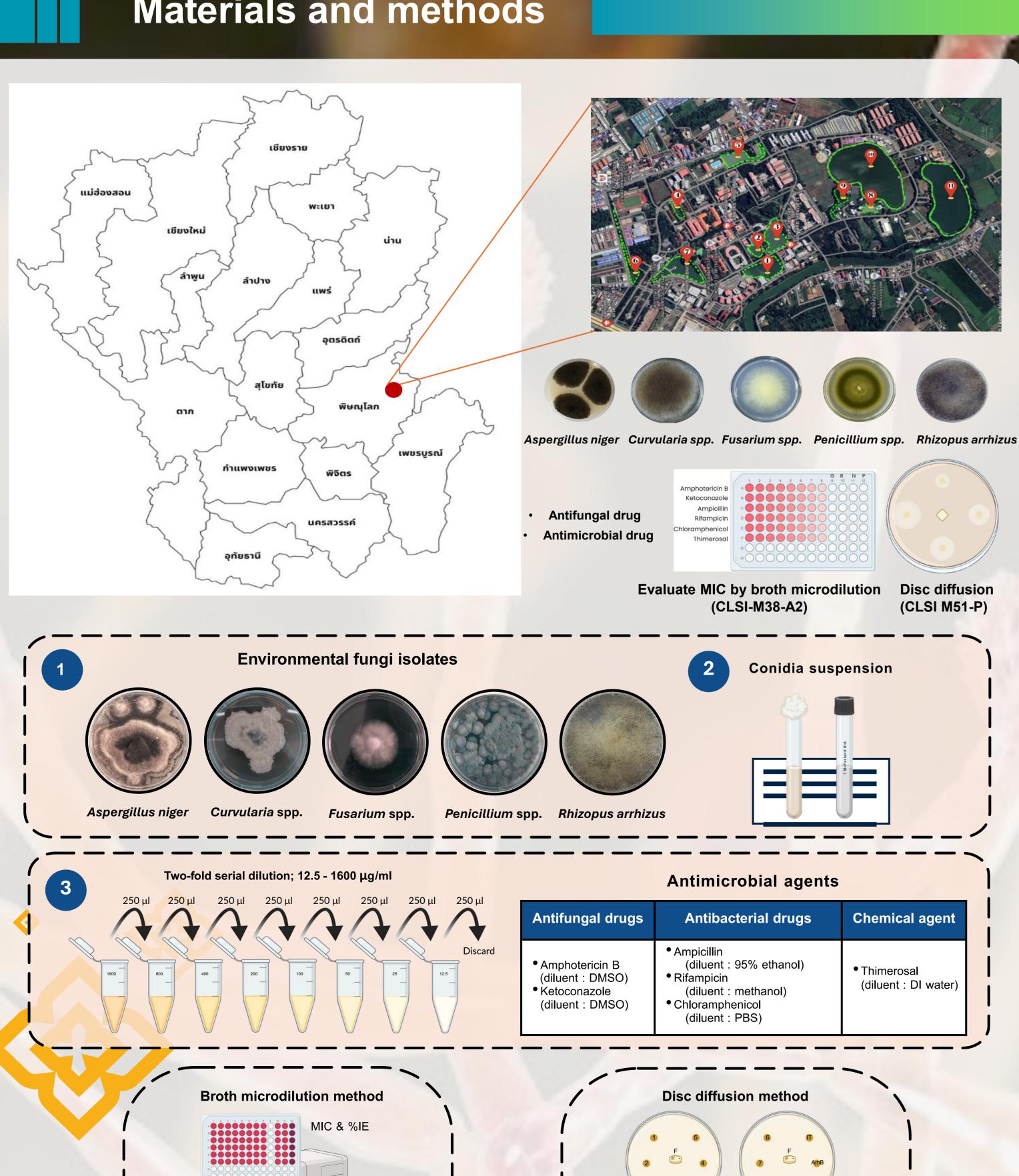
PC – absorbance of the positive control NC – absorbance of the negative control

– absorbance of the sample

To evaluate the fungicidal effect of antimicrobial agents against environmental fungi ,Including Aspergillus niger , Curvularia spp. , Fusarium spp., Penicillium spp. and Rhizopus arrhizus by broth microdilution method and verify by disc diffusion method.

Re-immerging and resistance?

Materials and methods



F: mycelial culture of fungal test

1-8: tested concentrations of antimicrobial agents

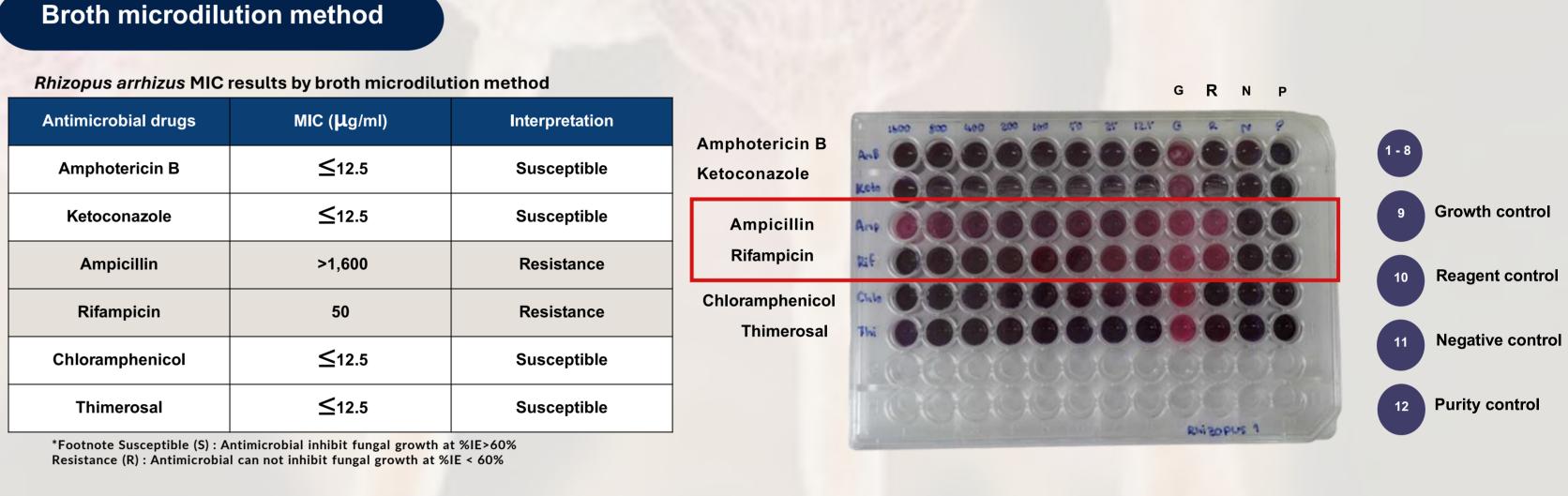
IT: Itraconazole (30 μg/mL)

Inhibition zone

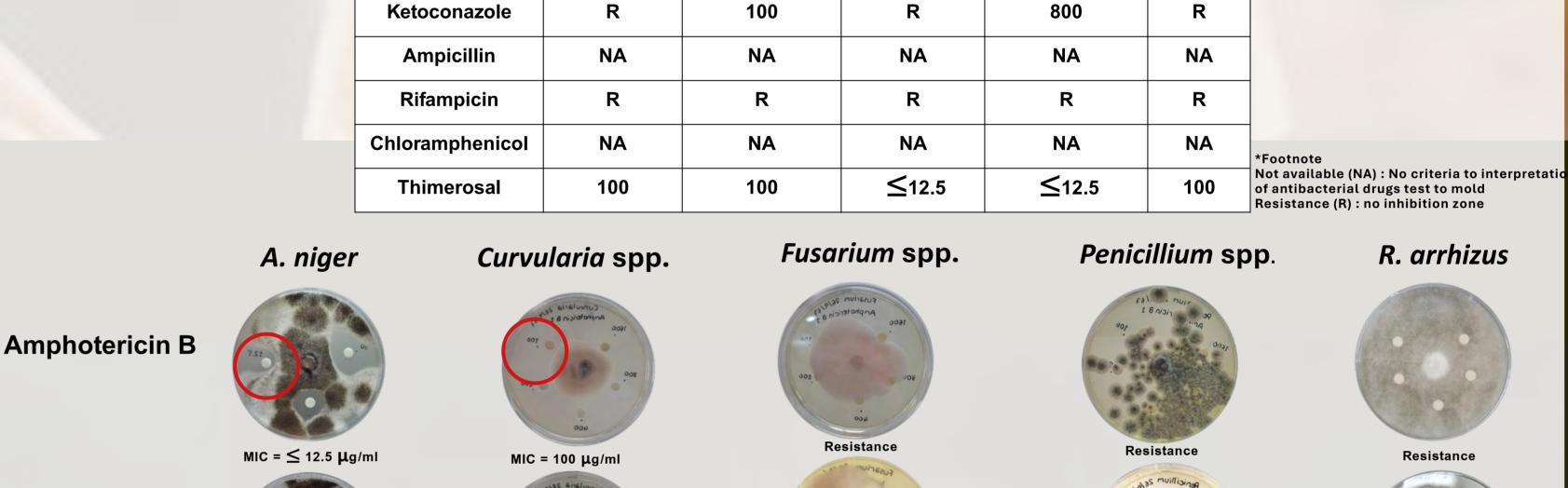
measurement

Control AmB: Amphotericin B (20 µg/mL)

Results

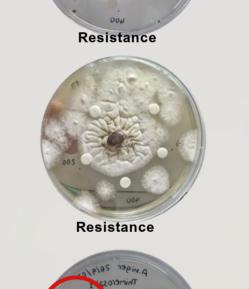


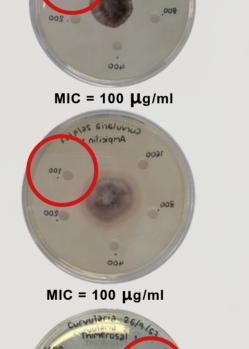
Disc diffusion method	Antimicrobial drugs / Fungi	A. niger (µg/ml)	Curvulria spp. (µg/ml)	Fusarium spp. (µg/ml)	Penicillium spp. (µg/ml)	R. arrhizus (µg/ml)
	Amphotericin B	≤ 12.5	100	R	R	R
	Ketoconazole	R	100	R	800	R
	Ampicillin	NA	NA	NA	NA	NA
	Rifampicin	R	R	R	R	R
	Chloramphenicol	NA	NA	NA	NA	NA

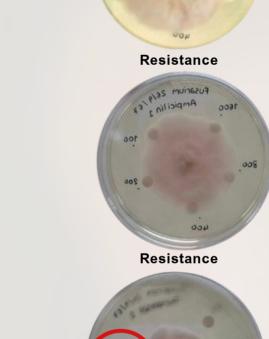




Thimerosal









Discussion and Conclusion

Rhizopus arrhizus showed growth and color change with resazurin, while the other four speciesdid not, possibly due to inconsistencies in conidia counts and growth rates. DMSO should also be diluted less than 10% concentration to prevent inhibiting fungal growth. (1)

The results of broth microdilution method indicated that R. arrhizus resistance to ampicillin, chloramphenicol, and rifampicin, with MIC values of >1,600, and 50 µg/mL, respectively. It was susceptible to amphotericin B, ketoconazole, chloramphenicol and thimerosal at a concentration of ≤ 12.5 µg/mL. The four remaining fungal species could not determine.

Further evaluation by disk diffusion method revealed that thimerosal has the best ability to inhibit the growth of all five fungal strains tested, followed by ketoconazole (60 %), amphotericin B (40 %), and ampicillin (20 %) respectively, and it was found that all fungi were resistant to chloramphenicol and rifampicin (100 %). Conversely, thimerosal showed great antifungal activity. (2) Further investigation for the toxicity focuses on narrowing the concentration ranges of antimicrobials agents. Their antifungal effects alone or in combination with natural compounds will be evaluated to develop a new strategies for alternative fungal treatments in the future.

References

- (1) Randhawa MA. The effect of dimethyl sulfoxide (DMSO) on the growth of dermatophytes. Nihon Ishinkin Gakkai Zasshi. 2006;47(4):313-8.
- (2) Rahimian A, Lakzaei M, Askari H, Dostdari S, Khafri A, Aminian M. In vitro assessment of Thimerosal cytotoxicity and antimicrobial activity. J Trace Elem Med Biol. 2023;77:127129.

Acknowledgement



