“Nen Dat” essential oil: *in vitro* antimicrobial activities and perspectives

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INTRODUCTION

“Nen dat” is a well-known resin in arts and certain industries as Damar batu. Intriguingly, it has been used as a therapeutic treatment in folkloric remedies in the north part of central Vietnam. This is believed to have the effect on prevention and treatment of post-delivery urogenital tract infections. “Nen dat” is also used when infants have signs of gastro-intestinal or respiratory problems.

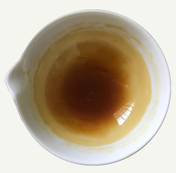
Previous study of Duong Thi Thanh Mai (2015) has shown that “Nen dat” is an oleoresin which may originally from Dipterocarpaceae family with the volatile essential oil and the non-volatile part separated by water distillation. Chemical analysis by gas chromatography coupled with mass spectrometry shows that “Nen dat” essential oil (NEO) contains sesquiterpenoids in which α-copaene, β-elemene, β-caryophyllene, caryophyllene oxide, and (+)spathulenol are major components. The non-volatile part of “Nen dat” on the other hand contains triterpenoids with the chemical structure of oleanane (β-amyrin), ursane (α-amyrin), lupane (lupeol) [4].

In order to go further on this research object, it is tempted to elucidate antimicrobial as well as antifungal activities of “Nen dat” *in vitro* before continuing on *in vivo* models.

MATERIALS

Nến Đất - Chai cục

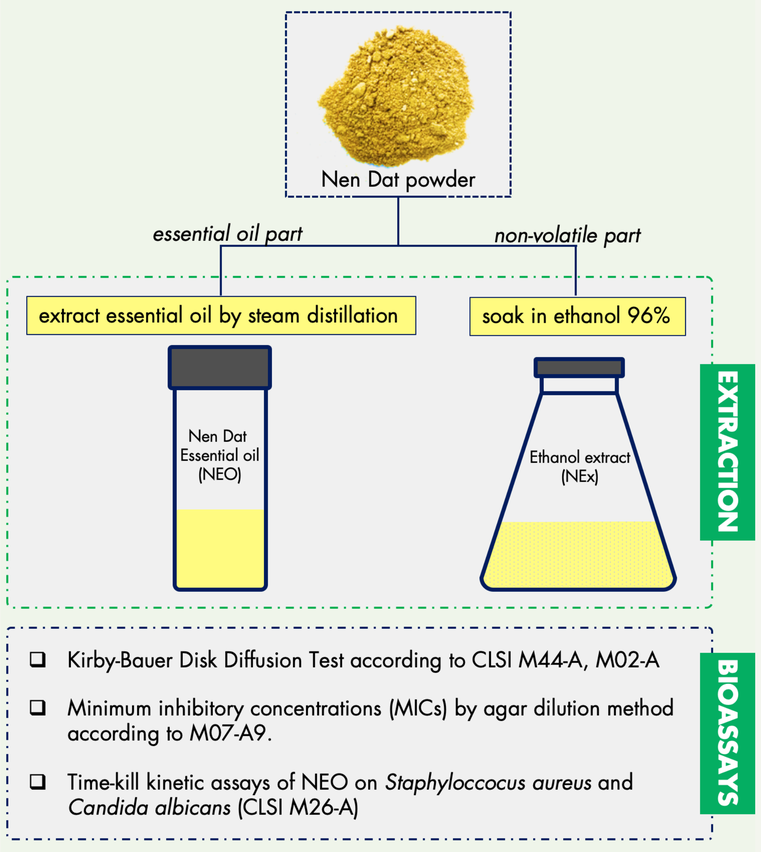
Nen Dat's powder



Ethanol extract

Nen Dat essential oil

METHODS



BIOASSAYS

- Kirby-Bauer Disk Diffusion Test according to CLSI M44-A, M02-A
- Minimum inhibitory concentrations (MICs) by agar dilution method according to M07-A9.
- Time-kill kinetic assays of NEO on *Staphyloccocus aureus* and *Candida albicans* (CLSI M26-A)

RESULTS

Diffusion assay

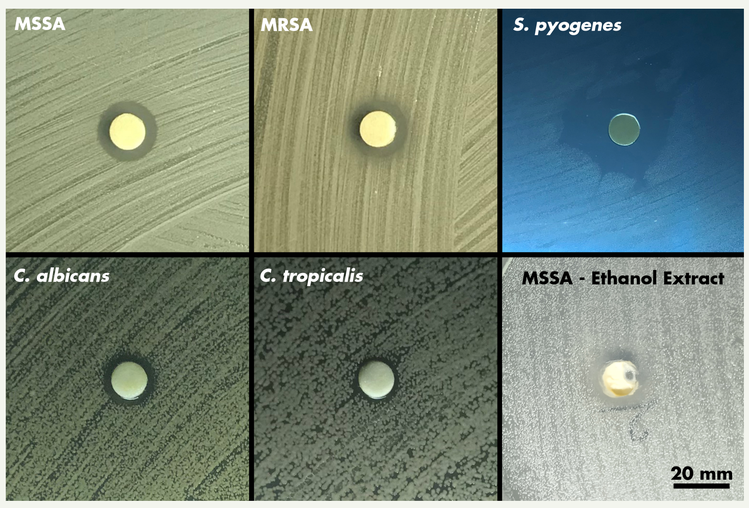


Table 1. The diameter zone of inhibition (DZI) of Nen Dat ethanol extract and essential oil (mm)

Bacteria/ Yeasts	NEx	NEO
S. aureus ATCC 29213 (MSSA)	**10,60**	**10,35**
S. aureus ATCC 43300 (MRSA)	0	**10,00**
Enterococcus faecalis ATCC 29212	n/a	**11,00**
Streptococcus pyogenes ATCC 19615	**18,19**	**18,00**
Candida albicans ATCC 10231	0	**9,28**
Candida tropicalis PNT20	0	**8,05**

The results show the effect of NEO on Gram positive bacteria and *Candida* spp., in which *Streptococcus pyogenes* is an important agent in the severe cases of postpartum infection. NEx preliminary shows certain effects on *S. pyogenes* and *S. aureus*.

MIC values

Table 2. The MIC values of Nen Dat ethanol extract and essential oil (µL/mL)

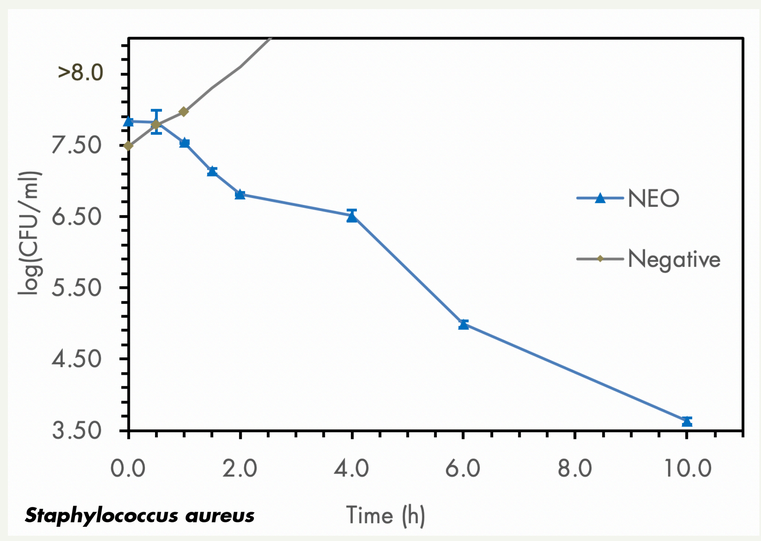
Bacteria/ Yeasts	NEx	NEO (HUP)	NEO (UMP)
S. aureus ATCC 29213	**>10**	**0.08**	**0.313**
S. aureus ATCC 33591 (MRSA)	n/a	**0.08**	**0.313**
E. faecalis ATCC 29212	n/a	**0.156**	**1.25**
Strep. pyogenes ATCC 19615	**<0.68**	**0.08**	**0.156**
C. albicans ATCC 10231	n/a	n/a	**5-10**
C. tropicalis PNT20	n/a	n/a	**10**
C. glabrata ND31	n/a	n/a	**5**
Candida sp. CNA821	n/a	n/a	**5-10**
Candida sp. CNA822	n/a	n/a	**10**
Candida sp. CNA100	n/a	n/a	**10**

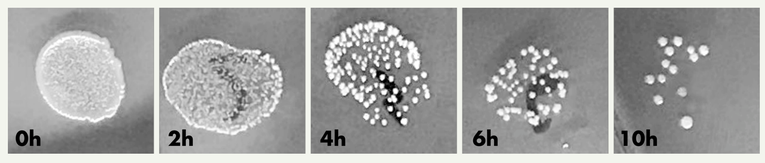
n/a: the test was not conducted

The average yield of NEO extraction was 0.4%. The minimum inhibitory concentration (MIC) on Gram positive bacteria and *Candida* strains are remarkable, varied from 0.08-0.156 µL/mL and 1.25 µL/mL, respectively for NEO (HUP), and 0.16-1.25 µL/mL and 5-10 µL/mL, respectively for NEO (UMP).

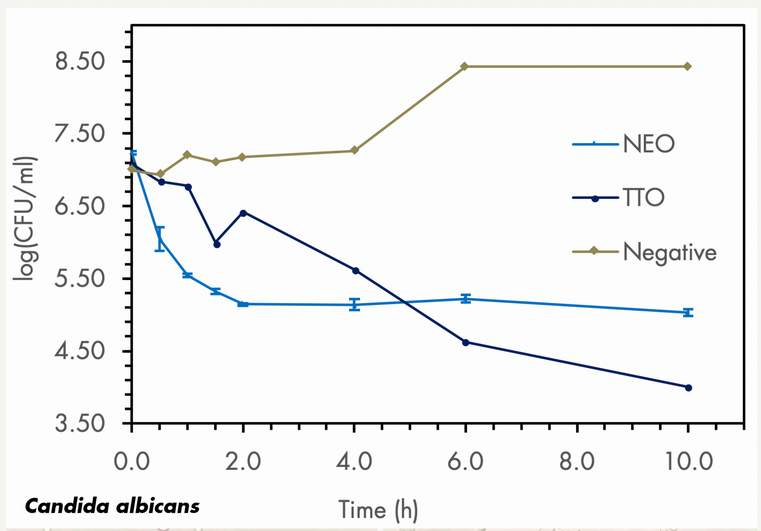
Time-kill kinetics observations of NEO on MRSA and *C. albicans* indicate bactericidal effect of NEO on MRSA at MIC (0.313 µL/mL). This can be clearly seen at 6h post treatment with NEO and the following hours afterwards. Inhibitory phenomenon was also seen on *C. albicans* but in a less effective manner compared to that on MRSA. Actually, *C. albicans* antimicrobial test usually express trailing effect, in the recent case, the number of *C. albicans* cultures remains from 2 to 10 hours post treatment.

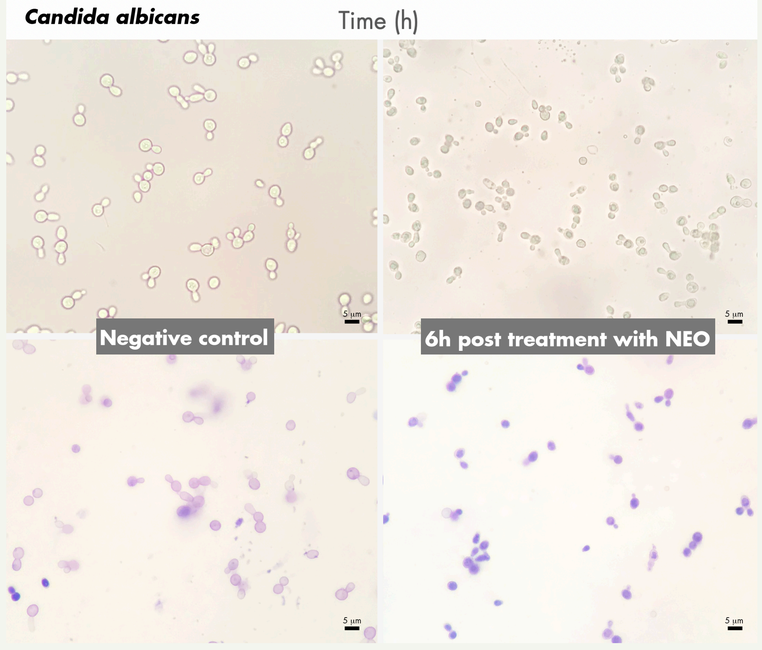
Time-kill kinetic assays





The density of *Staphylococcus aureus* ATCC 33591 increases remarkably as a logarithm phase and reaches at over 10^8 CFU/mL after 1.5 h in negative flask. The number of staphylococci in NEO sample decreases steadily during the observing period. At 10 h post treatment, NEO flask expresses the over 4-log downturn of bacteria, which indicates **bactericidal effect of NEO on MRSA** at MIC (0.313 µL/mL).





At 6h post treatment with NEO, *C. albicans* cells were damaged and stained in dark blue with methylthioninium chloride. Deformation of cellular wall were also seen in many cells.

REFERENCES

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CONCLUSION

This study brings data about antibacterial and antifungal activities of “Nen dat” essential oil, at the starting point of the study on Gram positive bacteria and *Candida* spp. Based on its special way of use in the community, “Nen dat” should be further investigated entirely and in combination with other antibiotics.

Thanks to the support from TRAC SEED FUND of The Training and Research Academic Collaboration Sweden – Vietnam (TRAC), this study is conducted to determine biological activities of NEO against bacterial and fungal organisms, focusing on urinary tract infectious pathogens.