Rachmani et al (2022)
Vol 10(2): 5929
https://doi.org/10.20884/1.api.2022.10.2.5929

Acta Pharmaciae Indonesia:
Acta Pharm Indo
E-ISSN 2621-4520

RESEARCH ARTICLE Open Access

Antifungal activity of *Calophyllum soulattri* leaf extract on fungal isolate of coconut neera

Eka Prasasti Nur Rachmani[1*], Hanif Nasiatul Baroroh[2], Rehana[3], Novia Ayu Rahmawati[1]

ABSTRACT

Background: Coconut neera is vulnerable to fungus contamination. Some plants, such as *Calophyllum soulattri*, can function as natural preservative with antifungal activity.

Objective: This study aimed to evaluate the antifungal activity of *C. soulattri* leaf extract on contaminant fungi in coconut neera as measured by minimum inhibitory concentration (MIC) and minimum killing concentration (MKC).

Methods: A dilution method was used to isolate the fungus isolate that contaminated coconut neera. MIC antifungal activity were then evaluated using the liquid dilution method.

Results: The results indicated that contaminant fungi found in coconut neera belonged the genus Penicillium. The MIC was 12.5%, while the MKC was 18.75%.

Conclusion: *C. soulattri* leaf extract has the potential to be developed as an antifungal for food preservation.

Keywords: *Calophyllum soulattri,* antifungal, minimum inhibitory concentration

Introduction

The quality of coconut sugar products is determined by the quality of coconut neera. Due to the high glucose content, coconut neera is vulnerable to fermentation by microorganisms such as fungi. The majority of sugar manufacturers use food preservatives, such as sodium metabisulfite, to solve this problem. However, practically coconut sugar makers usually fail to calculate the acceptable level of preservative for coconut neera, which may cause long-term health problems.

Calophyllum soulattri leaf is used empirically as natural preservative to inhibit the growth of fungi growth in coconut neera. *C. soulattri* leaf contains flavonoid, tannin, steroid, triterpene, and saponin [1]. This study aimed to determine the antifungal activity of *C. soulattri* leaf extract on contaminant fungi in coconut neera as measured by the minimum inhibitory concentration (MIC) and minimum killing concentration (MKC).

Methods

Plants and leaf extraction

C. soulattri leaves were obtained from Cilongok District, Banyumas Retency, Central Java, Indonesia. *C. soulattri* leaves were washed and dried in an oven at 70° C. The unprocessed *C. soulattri* leaves were then blended into powder. Five hundred gram powder was macerated using 4.5 liters of ethanol 96% for 3 x 24 hours. The filtrate was collected, evaporated and concentrated on waterbath until thick extract was produced.

Isolation and identification of fungi in coconut neera

The coconut neera were obtained from Cilongok District, Banyumas Regency without any preservative. This coconut neera was collected from dripping of coconut tree for 14 hours.

Fungi were isolated from damaged coconut neera using PDA (potato dextrose agar) medium on petri

[1]Laboratory of Pharmaceutical Biology, Department of Pharmacy, Jenderal Soedirman University, Purwokerto 53123, Indonesia

[2]Laboratory of Pharmacology, Department of Pharmacy, Jenderal Soedirman University, Purwokerto 53123, Indonesia

[3]Laboratory of Pharmaceutical Chemistry, Department of Pharmacy, Jenderal Soedirman University, Purwokerto 53123, Indonesia

*Corresponding author: Jl. DR. Soeparno Karangwangkal Campus, Jenderal Soedirman University, Purwokerto, Central Java 53123, Indonesia. E-mail: eka.rachmani2901@unsoed.ac.id

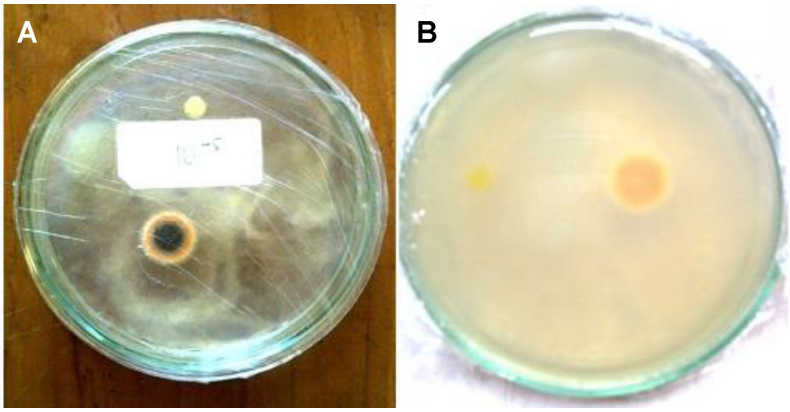

Figure 1. Fungal isolate. (A) Colony appearance from above, (B) colony appearance from the bottom of disc

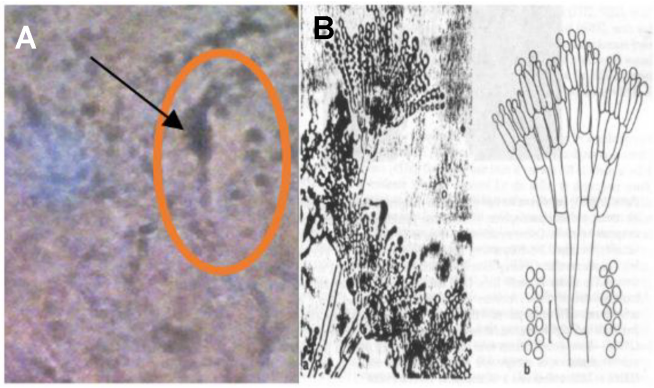

Figure 2. Fungi were observed microscopically. (A) fungal isolate, (B) *Penicillium* sp. Domsch dan Gams (1980)

dish. Fungal isolation was performed using dilution method from concentrations 10^{-1} to 10^{-5}. The dilution method was performed by inserting 1 ml sample of coconut neera into test tube and diluted with 9 ml sterile distilled water. Fungal isolation was performed using pour plate method by pouring 1 ml sample of diluted at concentrations 10^{-1} to 10^{-5} into 5 petri dishes, added with 15 ml liquid PDA, mixed until they were homogenous by rotating petri dish slowly on a table. The media stood for solidify, and the petri dishes were put upside down so that condensed water did not fall onto agar surface. All petri dishes were then wrapped and stored at room temperature for 3-7 days. The fungal colonies growing on the petri dishes were isolated for every different colony. The isolated colonies were purified by taking one inoculation loop of fungal colony and planted on PDA on petri dish and incubated at room temperature for 3-7 days.

The purified fungi were rejuvenated in PDA-contained slant test tube by taking one inoculation loop of purified fungi, to be put into PDA-contained slant test tube and incubated for 5-7 days. Fungal suspension was made by putting 5 ml sterile distilled water into pure fungi contained agar slant tube. Colony spore would be released from the medium, and then 1 ml fungal suspension was taken and put into empty test tube and added with 9 ml sterile distilled water for dilution at concentrations 10^{-1} to 10^{-3}.

The fungal isolates obtained were identified microscopically and macroscopically. Macroscopic properties were observed by observing the form and color of fungal colony and reverse of colony. Microscopic properties were observed by observing fungal isolates under microscope [2].

Antifungal activity test

Antifungal activity test was performed using liquid dilution method, including two stages: determining MIC (minimum inhibitory concentration) and MKC (minimum killing concentration). Potato dextrose broth (PDB) medium in each sterilized test tube was added with serially made *C. soulattri* extract and put into tube

Table 1. Fungal growth on PDB medium at varied concentrations of *C. soulattri* leaf extract

Replication	50%	25%	12.5%	6.2%	3.1%	1.5%	0.7%	Negative control	Media control
I	-	-	+	+ +	+ + +	+ + +	+ + + +	+ + + +	-
II	-	-	+	+ +	+ + +	+ + +	+ + + +	+ + + +	-
III	-	-	+	+ +	+ + +	+ + +	+ + + +	+ + + +	-

Note: - (clear, no fungal growth), + (slightly cloudy, there is fungal growth - quite a little), ++ (fairly cloudy, there is fungal growth - a little), +++ (cloudy, there is fungal growth - a lot), ++++ (very cloudy, there is fungal growth - plentiful)

for 1 ml and fungal suspension at dilution concentration 10^{-3} for 0.1 ml, and vortexed and incubated for 3 x 24 hours. The treatment was repeated thrice, and then observed and compared with control.

After 3 days of incubation, they were observed for MIC value by observing fungal growth on medium surface. Tube with the least fungal growth was taken for MKC value. Solution taken as MIC value was then used to determine MKC value by remaking varied extract concentrations from the range of MIC values obtained based on observation of fungal growth on medium surface. Liquid medium on the lowest concentration that did not show any fungal growth on medium surface after incubation was determined as MKC value.

Data analysis

The data were the extract's MIC and MKC values on fungi.

Results

Identification of fungi in coconut neera

Fungal was isolated from coconut neera that was retained for 14 days without any preservative. The isolated fungi were taken from the fungi growing in coconut neera at dilution concentration 10^{-5}. Macroscopic observation of fungal isolate was conducted on the color of greenish blue colony surface and yellow reverse of colony. Colony surface was soft like velvet or cotton. Microscopic observation as presented in Figure 1 found the characteristics of hypha's soft colony surface texture with septate, with fialid, conidiophores, single-cell spherical conidia.

The results of macroscopic and microscopic observation on the isolates conformed to the characteristics of *Penicillium* sp. Domsch and Gams (1980) stated that *Penicillium* sp. had the characteristics of colony colors of yellow, yellowish green, bluish green to brownish green. Colony surface was soft like velvet or sometimes like cotton, releasing yellow or hyaline exudate. Conidiophores formed vesicle at the end with varied count depending on the species, hypha had septate, had fialid, metula, spherical or elliptical one-septate conidia [3].

Macroscopic and microscopic identification of isolates was performed to examine the type of fungi contaminating the coconut neera. The identification results showed that the fungal isolates were expectedly *Penicillium* sp. This research result conforms to previous research that three general were identified in fungal isolation in damaged neera: *Penicillium* sp, *Aspergillus* sp. and *Curvularia* sp. The characteristics of *Penicillium* sp. were hypha with septate and branching mycelium and overall broom-like [4].

Antifungal activity test

The method used in the antifungal test was the liquid dilution method. Principally, this method used varied concentrations of *C. soulattri* leaf that were mixed into medium added with fungal suspension at concentration 10^{-3} and then incubated at room temperature and the results were observed through fungal growth on liquid medium surface in the tube. The parameters used in the test were MIC and MKC. The results of antifungal activity test on *C. soulattri* leaf showed that the extract was able to growth *Penicillium* sp.'s growth.

MIC value was determined by observing the fungal growth on medium surface. At concentrations 50% and 25%, the liquid medium was clear (Table 1). This shows that there was no fungal growth. At concentrations 50% and 25% *C. soulattri* leaf had very good antifungal activity. For MIC value, medium was

Table 2. Fungal growth at concentration range from minimum inhibitory concentration (MIC)

Replication	25%	21.8%	18.7%	15.6%	12.5%	6.2%	3.1%	Negative Control	Media Control
I	-	-	-	+	+	+ +	+ + + +	+ + + +	-
II	-	-	-	+	+ +	+ + +	+ + +	+ + + +	-
III	-	-	-	-	+	+ + +	+ + + +	+ + + +	-

Note: - (clear, no fungal growth), + (slightly cloudy, there is fungal growth - quite a little), ++ (fairly cloudy, there is fungal growth - a little), +++ (cloudy, there is fungal growth - a lot), ++++ (very cloudy, there is fungal growth - plentiful)

added with *C. soulattri* leaf at low concentrations of 6.25%, 3.125%, 1.56%, and 0.785. Fungal growth was identified with change in liquid medium's color to fairly cloudy, cloudy and very cloudy. At concentration 12.5% liquid medium was slightly cloudy, that the fungal growth was quite little. Therefore, the MKC value was 12.5%.

Table 2 shows that the MKC could be determined after MIC had been obtained. Variation of extract concentrations was performed at concentration with narrow range compared to determining MIC. At concentrations 25%, 21.875%, and 18.75%, liquid medium was clear. This shows that there was no fungal growth, At concentration 15.6% there was fungal growth on medium surface, while at concentrations 12.5%, 6.25%, 3.125%, the medium was fairly cloudy, cloudy and very cloudy, showing fungal growth. Based on the data, the MKC was at extract concentration 18.75%. The MKC of *C. soulattri* leaf shows a better result since the lower the MKC, *C. soulattri* leaf had better antifungal activity. The research on ethanol extract of leaf, root and stem partitioned with solvent petroleum ether, chloroform and ethyl acetate showed antibacterial activity [5].

Discussion

The active compounds in *C. soulattri* leaf are flavonoid, tannin, triterpene/steroid, and saponin [1]. Terpenoids, including triterpenoid and steroid, are bioactive compounds with antifungal function. These compounds may inhibit fungal growth, either through cytoplasmic membrane or disturbing fungal spore growth and development. Flavonoids work by inhibiting fungal cell division or proliferation. These compounds bind proteins in microtubules in cells and disturb mitosis function by causing fungal growth inhibition. Phenol compound serves as antifungal by denaturing protein bonds on cell membrane, leading to cell membrane lysis and allowing phenol to penetrate into cell core, causing fungi to not develop [6]. Saponin is antifungal with working mechanism of forming complex with sterol, that is to reduce the surface tension of sterol membrane, increasing permeability of mold and yeast cell membrane. Essential oil components may disturb the work of enzymes bound to cell membrane, disturbing fungal cell membrane's working activity [7].

Researches on the compounds contained in *C. soulattri* stark and stem stated there were derivative compound of coumarin, saulamarin, and derivative compounds of xanthone, filantrin and caloxanthone [8–10]. Compounds contained in a plant will qualitatively exist on other parts of that plant. Coumarin and xanthone have antifungal activity on *Aspergillus sp. and Candida albicans* [11]. The compounds in *C. soulattri* leaf show antifungal activity.

Conclusion

C. soulattri leaf has antifungal activity on contaminant fungi of *Penicillium* species. The MIC of *C. soulattri* leaf was at concentration 12.5%, and the MKC was 18.75%.

Funding

We express our gratitude to Jenderal Soedirman University with its grant fund for the research of Excellent Research scheme.

Author contributions

ER and R developed the research method; HNB and NAR assisted with data collecting; ER, NAR, R wrote the first script; HNB contributed to data analysis and data visualization; and all authors contributed to data

interpretation, manuscript editing, and manuscript approval.

Received: 31 May 2022
Revised: 30 July 2022
Accepted: 31 July 2022
Published online: 1 August 2022

References

1. Simanjuntak P, Septiana E. Aktivitas antioksidan ekstrak etanol kulit batang Calophyllum pulcherrimum, C. soulattri, dan C. teysmannii | Lembaga Ilmu Pengetahuan Indonesia [Internet]. 2019 [cited 28 Mar 2023]. Available: http://lipi.go.id/publikasi/aktivitas-antioksidan-ekstrak-etanol-kulit-batang-calophyllum-pulcherrimum-c-soulattri-dan-c-teysmannii/27983
2. Ristiari NPN, Julyasih KSM, Suryanti IAP. Isolasi dan identifikasi jamur mikroskopis pada rizosfer tanaman jeruk siam (Citrus nobilis Lour.) di kecamatan Kintamani, Bali. Jurnal Pendidikan Biologi Undiksha. 2019;6: 2019.
3. Compendium of soil fungi [Internet]. [cited 28 Mar 2023]. Available: https://agris.fao.org/agris-search/search.do?recordID=US201300137570
4. Suryandari KC. Uji Efektivitas Asap Cair Tempurung Kelapa Terhadap Jamur Dari Nira Rusak. Proceeding Biology Education Conference. Sebelas Maret University; 2010.
5. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of Calophyllum soulattri. Fitoterapia. 2002;73: 741–743. https://doi.org/10.1016/S0367-326X(02)00242-3
6. Hidayah N, Mustikaningtyas D, Bintari SH. Aktivitas Antibakteri Infusa Simplisia Sargassum muticum terhadap Pertumbuhan Staphylococcus aureus. Life Science. 2017;6: 49–54.
7. Ridawati R, Jenie BSL, Djuwita I, Sjamsuridzal W. Aktivitas antifungal minyak atsiri jinten putih terhadap Candida parapsilosis SS25, C. orthopsilosis NN14,C. metapsilosis MP27, dan C. etchellsii MP18. Makara Sains. 2011;15: 58–62.
8. Ee GCL, Mah SH, Teh SS, Rahmani M, Go R, Taufiq-Yap YH. Soulamarin, a new coumarin from stem bark of Calophyllum soulattri. Molecules. 2011;16: 9721–9727. https://doi.org/10.3390/molecules16119721
9. Mah SH, Ee GCL, Rahmani M, Taufiq-Yap YH, Sukari MA, Teh SS. A New Pyranoxanthone from Calophyllum soulattri. Molecules. 2011;16: 3999–4004. https://doi.org/10.3390/molecules16053999
10. Mah SH, Ee GCL, Teh SS, Rahmani M, Lim YM, Go R. Phylattrin, a new cytotoxic xanthone from Calophyllum soulattri. Molecules. 2012;17: 8303–8311. https://doi.org/10.3390/molecules17078303
11. Montagner C, de Souza SM, Groposoa C, Delle Monache F, Smânia EFA, Smânia A. Antifungal activity of coumarins. Z Naturforsch, C, J Biosci. 2008;63: 21–28. https://doi.org/10.1515/znc-2008-1-205