

Oleoresin *Baeckea frutescens* L. activity against *Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm.

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Abstract

This study aims to determine the oleoresin activity of *Baeckea frutescens* L. against the fungus *Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm. and to analyse its most active component. The research method used was ethanol solvent maceration extraction, and then fractionation was carried out using successive solvents, namely *n*-hexane, chloroform, and ethyl acetate. Then the *P. ostreatus* antifungal test, and analysis of the most active compound components using Gas Chromatography-Mass Spectrometry were applied. The results showed that *B. frutescens* oleoresin produced a yield of 11.41 % in ethanol extract. The most active antifungal activity was found in ethanol oleoresin soluble oleoresin with $IC_{(50)} = 177 \pm 02 \text{ mg} \cdot \text{L}^{-1}$. It contains 21 bioactive compounds consisting of 4 antifungal components, namely quinic acid, α -terpineol, linalool, and 2-cyclohexen-1-one.

Key words: antifungal, bioactive, extract, GC-MS, quinic acid.

Introduction

Indonesia has 4000 types of wood, and 80–85 % of them fall into durable classes III and IV. Types of wood that have a low durability class tend to be easily attacked by wood-destroying organisms. Those that most commonly attack tropical areas are wood rotting fungi. As a result of fungal attacks, the strength of the wood decreases because the cell wall components that strengthen the wood have been damaged. One of the wood rot fungi is *Pleurotus ostreatus* (Jacq.ex Fr.) P. Kumm. According

to Singh and Singh (2014) the fungus *P. ostreatus* attacks softwood. Overcoming attacks by wood-destroying fungi means preserving forests.

Overcoming wood damage by *P. ostreatus* can be done by using preservatives. Most of the preservatives used today are synthetic so they can cause environmental pollution. To prevent this, alternative materials are needed using biodegradable preservatives. The materials are easy to obtain and can be renewed. One of them is extractive substances contained in plants which have bioactive prop-

erties and are able to kill wood-destroying organisms (Setiawan et al. 2019). Extractive substances are secondary metabolic products of compounds that function ecologically and can be obtained from various types of plants. One of them is *Baeckea frutescens* L. which is a needle-leaved shrub from the Myrtaceae family (Supandi et al. 2019, Huang et al. 2023) which has the potential to be used for its leaves. They produce extractive substances in the form of essential oil. Previous research results stated that the leaves and twigs of *B. frutescens* produce essential oil which has anti-inflammatory, anti-oxidant, anti-bacterial, anti-fungal, anti-microbial and cytotoxic activity (Razmavar et al. 2014, Navanesan et al. 2015, Irma 2016, Nisa et al. 2017, Shahruzaman et al. 2019).

So far there have been no research reports regarding the activity of *B. frutescens* oleoresin against the fungus *P. ostreatus*. The results of research on oleoresin from *frutescens* are predominantly *Capsicum frutescens* L. (Do et al. 2021). It is a semi-solid extract consisting of resin and essential oils, obtained from extraction using organic solvents. Based on this background, research was carried out on the extraction of oleoresin from *B. frutescens* leaves, then its activity was tested on the fungus *P. ostreatus*, and the chemical content of the oleoresin was analysed. The aim of the research was to determine the activity of *P. ostreatus* and the oleoresin content of *B. frutescens*.

Material and Methods

Taking *B. frutescens* leaves and preparing leaf samples

B. frutescens leaves were obtained from forest areas in Kereng Bangkirai, Seban-

gau District, Palangka Raya City, Central Kalimantan (located at 2°16'14.595" to 2°06'18.8" S and 113°50'35.255" to 113°45'06.3" E). The leaves were harvested and then dried in the open. Drying until the leaf moisture content is 10–15 % (Zahra et al. 2019). The dried leaves were blended, then filtered by passing 40 mesh particles and held in 60 mesh. The leaf powder needed was 500 g.

Maceration

B. frutescens leaves with a dry weight of 250 g were macerated in ethanol at room temperature with a pressure of 1 atm for 24 h while stirring. The ratio of simplicia to solvent is 1:5 (v/v). The maceration process was repeated with stirring every three hours, for 15 min until a clear filtrate was obtained. The ethanol extract solution obtained was evaporated with a rotary evaporator at a temperature of 45–50 °C until the solvent was concentrated. The evaporated extract solution was placed in a 1000 mL glass above a batch of water at a temperature of 45–50 °C to evaporate the remaining solvent in the extract and obtain a thick extract (Setiawan et al. 2019).

Stratified fractionation of ethanol extract

50 mL of ethanol extract from *B. frutescens* leaf powder was put into a funnel separator for multilevel fractionation. Fractionation in stages, using the first solvent *n*-hexane 100 mL. This mixture was shaken for 10–15 min and left until separation occurred between the ethanol and *n*-hexane solvents. Fractionation of the *n*-hexane obtained. Next, the residue obtained was mixed with chloroform solvent. In the same way, separation is carried out and the chloroform and residue

fractions are produced. In the final stage, the resulting residue is mixed with ethyl acetate solvent. The multistage fractionation process is shown in Figure 1. The extract from each fraction was concentrated using a rotary evaporator to evaporate the remaining solvent and produce a thick extract (Setiawan et al. 2019).

The ethanol extract of 100 mL was subjected to multilevel fractionation with different solvents sequentially *n*-hexane, chloroform, and ethyl acetate. The fractionation process consists of a mixture of crude extract, solvent, and distilled water in a ratio of 1:2:2. The crude extract mix-

ture was shaken in a separatory funnel for 10–15 min and then left until synchronisation occurred between the dissolved extract and the crude extract which dissolved in ethanol. Each filtrate fraction obtained was evaporated using a rotary evaporator to evaporate the remaining solvent and produce a thick extract (Setiawan et al. 2019).

Cultivation of the fungus

The fungus used was *P. osteratus* which belongs to the group of wood-decaying fungi (Nakazawa et al. 2017). It was first

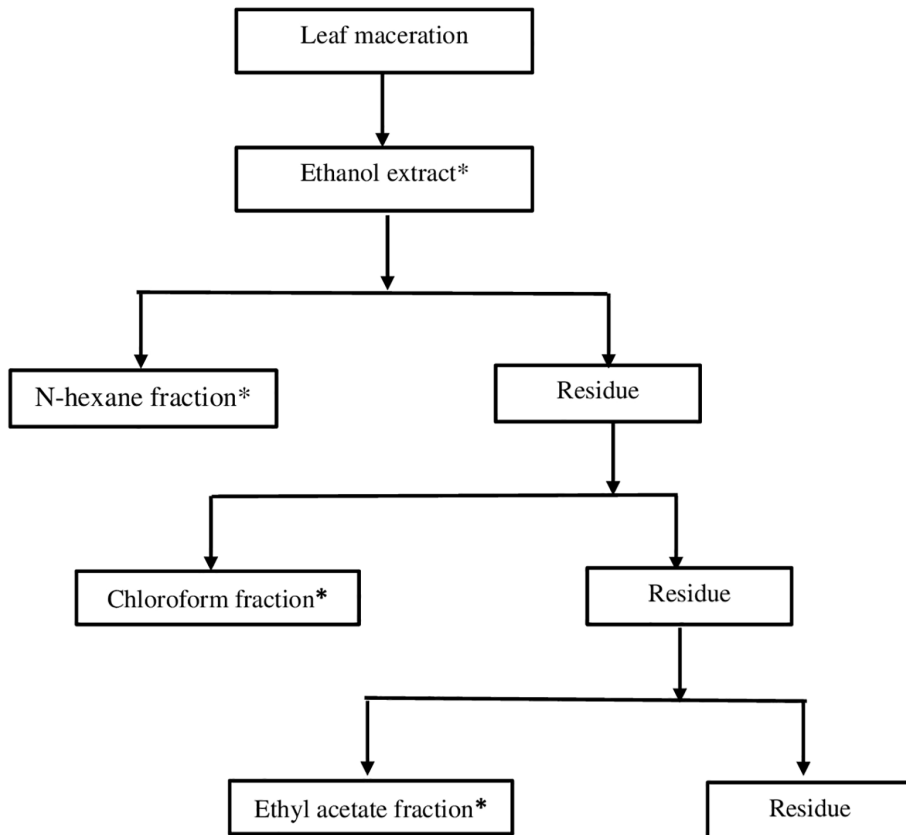


Fig. 1. Scheme of multistage oleoresin extraction using 4 solvents.

Note: * tested for fungus *P. osteratus*.

cultured on potato dextrose agar (PDA) growth medium for 7 days. The composition of the growth medium is as follows: 1 L contains 50 g glucose, 120 g onion extract, 50 g glucose, 0.3 g K_2HPO_4 , 0.2 g $MgSO_4 \cdot 7H_2O$, 5 g polypeptone and 30 g agar powder at pH 6.0.

Anti-fungal activity testing

Testing of all oleoresins, which were obtained from maceration and fractionation was realised. The treatments were variations in the concentration of extractive substances as follows: 0, 50, 100, 250, 500, and 1000 $mg \cdot L^{-1}$. Previously the Petri dish was autoclaved for 15 min at a temperature of $\pm 120^\circ C$ with a pressure of 1 atm. Poured with media and oleoresin according to the treatment, each treatment was repeated 3 times and planted with *P. osteratus* mushrooms. Next, it was incubated at $25^\circ C$ for 7 days in a dark room. The inhibitory activity of fungal growth by extractive substances was carried out at the end of the incubation period, by measuring the growth diameter and comparing it with the growth of control mycelium. The basis for determining anti-fungal activity uses the formula (1) (Kamaruzzaman et al. 2021).

$$\text{Percent inhibition} = (C - T) \cdot C^{-1} \cdot 100, \quad (1)$$

where: T is the area of mycelium in the treatment petri dish, and C is the area of mycelium in the control Petri dish.

Data analysis

Data on the percentage of inhibition of fungal growth were analysed with probit to determine the value of $IC_{(50)}$. Calculations using the SPSS 17.0 program. A compound was declared active if it had an $IC_{(50)}$ value $< 1000 \text{ mg} \cdot L^{-1}$ and inactive if it had an $IC_{(50)}$ value $> 1000 \text{ mg} \cdot L^{-1}$ (Meyer et al. 1982).

Gas chromatography-mass spectrometry (GC-MS) analysis

A total of 0.5 mg of oleoresin *B. frutescens* which had the highest inhibition was carried out by the GC-MS test. The results were analysed and adjusted using the WILLEY7.LIB database. GC-MS brand is Shimadzu; Type: GCMS QP2010 Plus. The analysis was carried out at the Commodity and Environmental Testing Laboratory, Center for Industrial Research and Standardisation. Banjarbaru South Kalimantan Indonesia.

Results and Discussion

Oleoresin antifungal *B. frutescens*

As a result of maceration of the leaves of *B. frutescens* with ethanol solvent it was obtained 11.41 % oleoresin, 2.55 % *n*-hexane, 2.01 % chloroform, and 0.50 % ethyl acetate. The differences in yields produced were due to differences in the polarity of each solvent. *B. frutescens* oleoresin which is soluble in several solvents was then tested for the activity of the *P. ostreatus* fungus. The results of the fungal growth inhibition test are shown in Table 1 and the growth of fungal mycelium at several oleoresin concentrations is shown in Figure 2.

Table 1. $IC_{(50)}$ value of *B. frutescens* oleoresin against *P. ostreatus* fungus.

No	Soluble oleoresin	$IC_{(50)}$, $mg \cdot L^{-1}$	Classification*
1	Ethanol	177 ± 0.21	Active
2	<i>n</i> -hexane fraction	242 ± 0.15	Active
3	Chloroform fraction	233 ± 0.61	Active
4	Ethyl acetate fraction	270 ± 0.14	Active

Note: * Classification is according to Meyer et al. (1982).

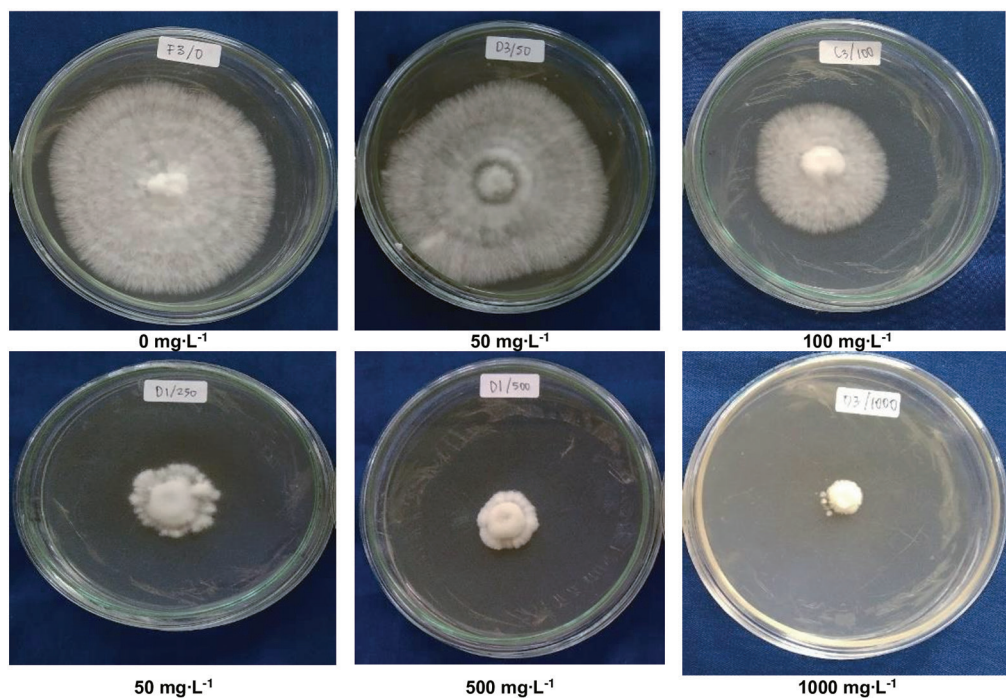


Fig. 2. Effect of *B. frutescens* oleoresin concentration on the growth of *P. ostreatus* mycelium (photo by L. R. Sitorus).

Table 1 displays the results of *B. frutescens* oleoresin activation test on the growth of *P. ostreatus* mycelium with $IC_{(50)} = 177 \pm 0.2$ to 270 ± 0.14 $mg \cdot L^{-1}$. As well as the anti-fungal activation of *B. frutescens* oleoresin, all of them are classified as active because $IC_{(50)} \leq 1000$ $mg \cdot L^{-1}$ (Meyer et al. 1982). However, the ethanol soluble oleoresin extract was the most active of the four. This is because the ethanol soluble oleoresin extract has the lowest $IC_{(50)} = 177 \pm 0.2$ $mg \cdot L^{-1}$ and was able to inhibit the mycelium of *P. ostreatus* fungus compared to the four oleoresin extracts. The research results of Wu et al. (2021) stated that the ethanol extract of *Fagus crenata* Blume wood was able to inhibit the delignification process and hydrolytic enzyme reactions of *P. ostreatus*. The research results of Fakhrudin et al. (2015), Hastuti et al. (2018), Dewi et al. (2019). stated ole-

oresin from *Ginger* rhizomes and *Polygonum minus* Huds. is with a higher phenol content, so it can inhibit the growth of *Trichophyton rubrum* Sabour.

Content of oleoresin compound *B. frutescens* which dissolves in ethanol

B. frutescens oleoresin in ethanol extract had the most active *P. ostreatus* growth inhibition activity than the *n*-hexane fraction, chloroform fraction, and ethyl acetate fraction. So, the selection was made to remove the content of the oleoresin compound using GC-MS analysis which is presented in Figure 3.

Analysis of the components of the oleoresin compound *B. frutescens* in the ethanol extract showed 21 compounds and the literature review which are presented in Table 2.

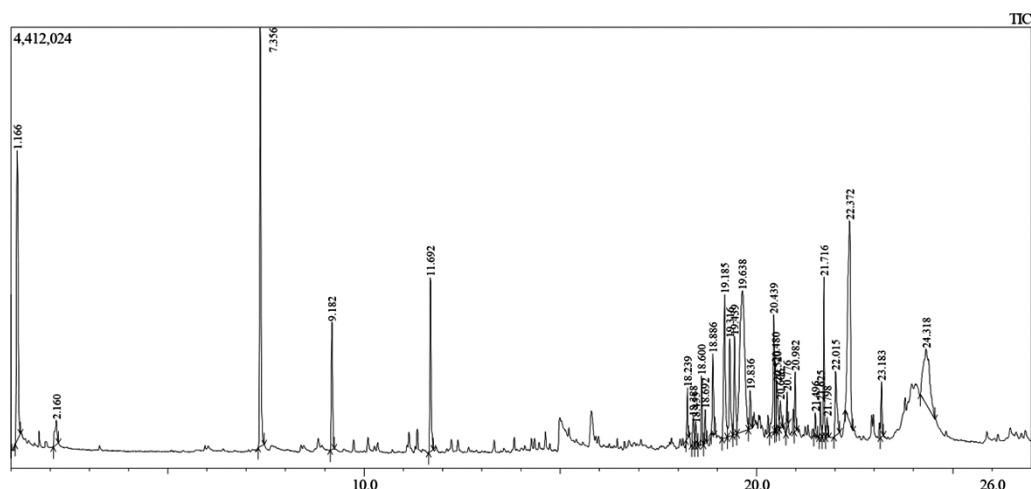


Fig. 3. Chromatogram of *B. frutescens* oleoresin in ethanol extract.

Note: Retention time (RT) 2.160 = propanoic acid; RT 7.356 = 1,8-cineole; RT 9.182 = linalool; RT 11.692 = α -terpineol; RT 18.239 = (-)-caryophyllene oxide; RT 18.388 = quinic acid; RT 18.451 = β -selinene; RT 18.691 = 3,4-dimethoxyphenyl propionamide; RT 19.188 = 3-methoxymethyl-2,5,5,8a-tetramethyl-6,7,8,8a-tetrahydro-5h-chromene; RT 19.440 = 2-cyclohexen-1-one; RT 20.519 = anthracene, 9-trimethylsilyl; RT 20.604 = pluchidiol; RT 20.775 = (R)-2-benzyl proline methylamide; RT 20.98 = ergostane-3,5,6,12,25-pentol, 25-acetate; RT 21.497 = 4-methoxy-3-(methoxymethyl)phenol; RT 21.624 = 4,4,8-trimethyl-tricyclo [6.3.1.0 1,5] dodecane-2,9-diol; RT 21.797 = 2-propenoic acid; RT 22.009 = phi-aspidinol; RT 22.372 = 1-butanone; RT 23.183 = Methan; RT 24.317 = Urs-12-en-28-al.

Table 2. GC-MS of *B. frutescens* oleoresin in ethanol extract.

Compound	SI	Content, %	Compound formula	Bioactive (references)
Propanoic acid	-	2.274	$C_3H_6O_2$	Antimicrobial (Zhang et al. 2011)
1,8-cineole	90	5.499	$C_{10}H_{18}O$	Antiinflammatory (Santos and Rao 2000)
Linalool	82	1.912	$C_{10}H_{18}O$	Antifungal (Dias et al. 2018)
α -terpineol	86	3.047	$C_{10}H_{18}O$	Antifungal (An et al. 2019)
(-)-caryophyllene oxide	80	0.822	$C_{15}H_{24}O$	Anticancer and analgesic (Fidy et al. 2016)
Quinic acid		0.214	$C_7H_{12}O_6$	Antifungal (Ma and Ma 2015)
β -selinene		0.224	$C_{15}H_{24}$	Antioxidant (Chandra et al. 2017)
3,4-dimethoxyphenyl propionamide		0.825	$C_{10}H_{15}NO_2$	Antibodies (Fernández-Martínez et al. 2001)
3-methoxymethyl-2,5,5,8a-tetramethyl-6,7,8,8a-tetrahydro-5h-chromene		14.564	$C_{15}H_{24}O_2$	
2-cyclohexen-1-one		0.842	C_6H_8O	Antifungal (Shakil et al. 2013)

Compound	SI	Content, %	Compound formula	Bioactive (references)
Anthracene, 9-trimethylsilyl		1.082	C ₁₅ H ₁₁ Cl	Anticancer (Sirimanne et al. 1991)
Pluchidiol		0.845	C ₅ H ₁₂ ClNO ₂	
(R)-2-benzyl proline methylamide		0.635	C ₁₂ H ₁₅ NO ₂	
Ergostane-3,5,6,12,25-pentol, 25-acetate		0.272	C ₃₀ H ₅₂ O ₆	
4-methoxy-3-(methoxymethyl) phenol		0.383	C ₉ H ₁₂ O ₃	Antimicrobial and antiinflammatory (Manivannan and Shopna 2017)
4,4,8-trimethyl-tricyclo [6.3.1.0 1,5] dodecane-2,9-diol		0.175	C ₁₀ H ₁₉ NO	
2-propenoic acid		0.195	C ₃ H ₄ O ₂	Antimicrobial (Murdoch et al. 2007)
phi-aspidinol		2.179	C ₁₂ H ₁₆ O ₄	
1-butanone		33.435	C ₄ H ₈ O	
Methan		1.754	CH ₄	Antimicrobial (Rawat and Singh 2019)
Urs-12-en-28-al		1.589	C ₃₀ H ₄₈ O	

Note: SI is similarity index.

Table 2 explains the components of the dominant *B. frutescens* oleoresin compound, namely 1,8-cineole, 3-methoxymethyl-2,5,5,8a-tetramethyl-6,7,8,8a-tetrahydro-5h-chromene and 1-butanone. The 1,8-cineole gives the characteristic aroma of fresh camphor, and spicy and anti-fungal properties to the oleoresin of *B. frutescens* (Tadtong et al. 2015, Kim et al. 2018). In the ethanol extract, 4 active antifungal compound components were obtained, namely α -terpineol, linalool, 2-cyclohexen-1-one and quinic acid (Shakil et al. 2013, Ma and Ma 2015, Kong et al. 2019, Li et al. 2022). The dominant compounds contained were from the terpenoid group. The most abundant terpenoids are monoterpenes and sesquiterpenes with amounts of C10 and C15. The two types of terpenoids had differences in boiling points which can affect gas chromatography, compounds with low boiling

points will come out first into the detector. This is possible because a lower boiling point causes the compound to evaporate more easily so the retention time is faster. The retention time of each compound component is determined by the boiling point of the compound component (Harianingsih et al. 2017). Due to the low similarity index (SI) of standard mass spectra from the NIST12, NIST62 and WILEY229 tool libraries cannot be used. Supporting data through searching mass spectra from previous research results were found as well. So further SI analysis was carried out with a literature study of fragmentation patterns. The compounds 1,8-cineole, linalool, α -terpineol, (-)-caryophyllene oxide resulted from GC-MS analysis in Table 2. According to the similarity index value with the results of GC-MS analysis of *B. frutescens* essential oil it is the same as the similarity index value resulting

from research using essential oils from *B. frutescens* leaves from research by Saad et al. (2021), Jemi et al. (2021) and Tam et al. (2004). The SI of a compound showed that it is close to 100 %, indicating that it is getting closer to the compound standard (Fitriani et al. 2020).

Based on the results of GC-MS analysis and literature searches, the four compounds are anti-fungal. Of them, the more dominant is quinic acid based on the number of -OH functional groups. Because the -OH groups are reactive and capable of damaging fungal cell walls and cannot be eliminated by fungal enzymatic reactions. So, the non-functioning of lignolytic enzymes results in disrupted fungal growth and ultimately death (Shakil et al. 2013, Ma and Ma 2015, Kong et al. 2019, Broda 2020, Li et al. 2022).

Conclusion

B. frutescens oleoresin produced the highest yield in ethanol extract, followed by the *n*-hexane, chloroform, and ethyl acetate fractions. Ethanol-soluble oleoresin was the most active in inhibiting the growth of *P. ostreatus*. It also contains anti-fungal compounds, namely quinic acid, α -terpineol, linalool and 2-cyclohexen-1-one.

The research results recommend that *B. frutescens* oleoresin can be processed and used as an environmentally friendly wood preservative, especially against attacks by the fungus *P. ostreatus*. This can reduce the use of synthetic preservatives.

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