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Antibacterial and Antioxidant Activities of *Cutibacterium acnes* on *Jatropha gossypifolia* leaves and *Pommetia pinata* Bark

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Abstract: *Cutibacterium acnes* is a bacterium that can cause inflammation of the skin tissue and lead to acne. Inhibitory activity treatment can be carried out using natural compounds unique to Indonesia as a tropical country, namely *Pometia pinnata* stem bark and red *Jatropha gossypifolia* leaves. *Pometia pinnata* stem bark and red *Jatropha gossypifolia* leaves are known to have the potential for antibacterial activity. This study aims to combine the two plants as anti-bacterial agents against *Cutibacterium acnes* and as antioxidants. The method used in this study was maceration, employing ethanol and ethyl acetate solvents for antibacterial and antioxidant activity tests, specifically paper disc diffusion and DPPH assays. The results showed that *J. gossypifolia* and *Pometia pinnata* leaf extracts contain flavonoids, steroids, terpenoids, alkaloids, and tannins. The antibacterial activity of a mixture of ethanol extracts from *J. gossypifolia* and *Pometia pinnata* leaves against *Cutibacterium acnes* bacteria was strong, categorized at a concentration of 25%. Additionally, there was no significant difference in the antioxidant capacity between the mixture of ethanol and ethyl acetate extracts. The antioxidant capacity value was approximately 53% AEAC at a concentration of 500 ppm.

Keywords: *Cutibacterium acnes, Jatropha gossypifolia, Pommetia pinata,* DPPH

***Abstrak*:** *Cutibacterium acnes merupakan bakteri yang dapat menyebabkan peradangan pada jaringan kulit dan memicu timbulnya jerawat. Perlakuan terhadap aktivitas penghambatan dapat dilakukan dengan menggunakan senyawa alami khas Indonesia sebagai negara tropis, yaitu kulit batang Pometia pinnata dan daun Jatropha gossypifolia merah. Kulit batang Pometia pinnata dan daun Jatropha gossypifolia merah diketahui mempunyai potensi aktivitas antibakteri. Penelitian ini bertujuan untuk menggabungkan kedua tanaman tersebut sebagai anti bakteri terhadap Cutibacterium acnes dan sebagai antioksidan. Metode yang digunakan dalam penelitian ini adalah maserasi dengan menggunakan pelarut etanol dan etil asetat untuk uji aktivitas antibakteri dan antioksidan, khususnya difusi cakram kertas dan uji DPPH. Hasil penelitian menunjukkan bahwa ekstrak daun J. gossypifolia dan Pometia pinnata mengandung senyawa flavonoid, steroid, terpenoid, alkaloid, dan tanin. Aktivitas antibakteri campuran ekstrak etanol daun J. gossypifolia dan daun P. pinnata terhadap bakteri Cutibacterium acnes termasuk kuat, dikategorikan pada konsentrasi 25%. Selain itu, tidak terdapat perbedaan yang signifikan pada kapasitas antioksidan antara campuran ekstrak etanol dan ekstrak etil asetat. Nilai kapasitas antioksidan sekitar 53% AEAC pada konsentrasi 500 ppm.*

Kata kunci: *Cutibacterium acnes, Jatropha gossypifolia, Pommetia pinata, DPPH*

INTRODUCTION

Acne is a chronic skin disease that occurs due to inflammation of the sebaceous glands. The causes of its occurrence include excessive secretion of sebum, abnormal proliferation, hormonal factors, genetics, and lifestyle (Mawardi *et al.* 2021). This multifactorial disease affects almost all ages, but it is more likely in women. Research on the prevalence and incidence of acne over a ten-year period concluded that the percentage of potential acne sufferers was aged 10-19 years (Wang *et al.* 2022). The mechanism that occurs is related to the interaction of microbes on the skin and the host's immunity against these microbes (Lee *et al.* 2019).

Cutibacterium acnes, commonly associated with acne, is a gram-positive bacterium in the phylum Actinobacteria. Several mechanisms explain the role of these bacteria in acne, including augmentation of lipogenesis, formation of comedones, and host inflammation through lipase, protease, hyaluronidase, and CAMP activity. In the lipogenesis process, *C. acnes* causes the sebaceous glands to synthesize lipid droplets and triacylglycerol. Moreover, *C. acnes* produces oxidized squalene and free fatty acids, which contribute to increased sebum production.

Additionally, *C. acnes* activates IGF-1/IGF-1 receptors, leading to increased filaggrin expression. This elevation affects levels of integrin-α3, -α6, and vβ6, subsequently influencing keratinocyte proliferation and differentiation. This sequence leads to comedo formation, stimulates ROS production from keratinocytes to eliminate bacteria, and induces inflammation (Esmael *et al.* 2020; Lee *et al.* 2019)

Treatment often involves the use of antibiotics. However, the effectiveness of treatment can be hindered by the presence of *P. acnes* strains that are resistant to antibiotics. The extensive use of antibiotics to treat acne may result in the development of *P. acnes* strains with cross-resistance to different antibiotics, potentially impacting acne and other diseases (Lee *et al*. 2019; Esmael *et al.* 2020). Therefore, treatment using natural ingredients presents itself as one possible solution.

Several typical Indonesian plants exhibit antibacterial activity. *Pometia pinnata*, a native Papuan plant, serves various purposes (Nugrahani *et al.* 2021), including antidiabetic effects (Sukiman *et al.* 2018), antimicrobial properties (Fajrina *et al.* 2020), antioxidant activity (Hajar *et al.* 2021; Hasan *et al.* 2022) and contains various secondary metabolites like flavanols (Utari *et al.* 2019), saponins (Mohammad *et al.* 2012), and brassicasterol (Herru *et al*. 2021). These compounds collectively contribute to *Pometia pinnata*'s biological activities. A specific component of *Pometia pinnata*, its stem bark, demonstrates antibacterial activity against *Staphylococcus aureus* and has been examined for potential antiseptic properties. Consequently, this plant also holds potential to inhibit other acne-causing bacteria, particularly *Cutibacterium acnes*.

Another plant sharing similar potential is *Jatropha gossypiifolia*. This plant exhibits diverse bioactivities (Félix-Silva *et al.* 2014) serving as an antidiabetic agent (Granados *et al.* 2015). This plant has been investigated across various parts (Drafor *et al.* 2021) and has shown efficacy against various types of bacteri (Jeff-Agboola & Onifade 2016), (Nugraha *et al*. 2021) (Semuel Torokano n.d.). Hence, *Jatropha gossypiifolia* is a candidate for inhibiting *Cutibacterium acnes*, the bacteria responsible for causing acne.

Both of these plants are recognized for their antibacterial effects against *C. acnes* bacteria (Kurniati *et al.* 2022) and *Staphylococcus epidermidis* (Rossalinda *et al.* 2021). This potential could be harnessed to prevent acne development by acting against *C. acnes*. Consequently, we embarked on a study of both *Pometia pinnata* and Jatropha gossypiifolia. Extracts from each plant will be obtained using the same solvent and subsequently assessed individually as well as in combination. This combined approach is an innovative avenue of research that aims to leverage the synergistic effects of these two plants.

MATERIALS AND METHODS

Materials

The materials used in this study included distilled water (H_2O), a pure culture of *Cutibacterium acnes*, dimethyl sulfoxide (DMSO), red Jatropha leaves (*Jatropha gossypifolia* L.), *Pometia pinnata* stems (*Pometia pinnata*), ethanol (C_2H_5OH), ethyl acetate ($C_4H_8O_2$), Mueller-Hinton agar (MHA), Nutrient Agar (NA), 0.9% physiological NaCl, *n*-hexane (C_6H_{14}), paper discs, ethanol, tetracycline HCl, and sterile water

Instrumentation

The tools employed in this study consisted of the UV-Vis Spectronic 20D+ spectrophotometer (Thermo Electron Corporation, United States of America), Heraeus incubator (Thermo Scientific, United States of America), rotary evaporator, Esco Isocide 16444-1 laminar airflow cabinet (Esco, United States of America), acid cabinet, Memmert GmbH oven, GEA YX28OD autoclave (Japan), Kern ABJ 220-4M analytical balance (Germany), Velp Scientifica Wixard vortex mixer (Italy), Maspion S-300 electric stove (Indonesia), Biorad micropipette, blender, glassware, drip plate, microtip, scissors, bulb, tweezers, spatula, sterile tubes, and bottle racks.

Extraction

The extraction was conducted using the maceration method. Each sample (*Jatropha gossypifolia* and *Pometia pinnata*) underwent maceration with two different solvents, namely ethanol and ethyl acetate. The resultant extracts were obtained as dense substances and were ready for utilization.

Creating Combinations and Extract Concentrations

The acquired crude extracts encompassed *Jatropha gossypifolia* ethanol extract, *Jatropha gossypifolia* ethyl acetate extract, *Pometia pinnata* ethanol extract, and *Pometia pinnata* ethyl acetate extract. Subsequently, each type of solvent-matched extract was combined, including ethanol extract of *Jatropha gossypifolia* and a 50:50 combination (ethanol extract of *Jatropha gossypifolia* and *Pometia pinnata*). The same combination approach was applied to ethyl acetate extracts in both samples. Six samples were created, each maintaining consistent concentration variations at 25%, 20%, 15%, 10%, and 5% using ethanol and DMSO solvents.

Media Preparation

Nutrient Agar (NA) Media Preparation

The agar media was prepared by dissolving 1.38 grams of nutrient agar in heated distilled water. The resulting media solution was autoclaved for 15 minutes and then poured into sterile petri dishes.

Mueller-Hinton Agar (MHA) Media Preparation

Mueller-Hinton media was produced by dissolving 1.52 grams of Mueller-Hinton agar in heated distilled water. After thorough dissolution, the media solution was sterilized in an autoclave for 15 minutes. Following sterilization, the media solution was poured into sterile petri dishes**.**

Bacterial Revival

A pure isolate was streaked onto the surface of nutrient agar media and then incubated for 48 hours at 37ºC.

Preparation of McFarland Solution and Bacterial Suspension

A 0.5% McFarland solution was prepared by mixing 0.05 mL of 1% barium chloride ($BaCl_2$) solution and 9.95 mL of 1% H_2SO_4. The solution was homogenized, and its turbidity was measured at a wavelength of 625 nm using visible spectrometry. This solution served as a reference for the bacterial suspension. The bacterial suspension was generated by transferring one to two loops of bacteria to 10 mL of physiological NaCl. The suspension was homogenized and compared with the 0.5% McFarland solution.

Antibacterial Activity Test

The antibacterial activity of sample extracts was assessed using the disk diffusion method with Mueller-Hinton Agar (MHA) medium. A volume of 150 µL of bacterial suspension was placed onto the Mueller-Hinton agar medium and allowed to solidify. Paper discs were soaked for 15 minutes in each of the following: negative controls (DMSO), various sample concentrations, and a positive control (Tetracycline HCl). Subsequently, each paper disc was placed onto the Mueller-Hinton media within specified areas. The media were then incubated at 37°C for 48 hours, and the inhibition zones were observed.

DPPH Antioxidant Assay

The assessment of antioxidant activity in sample extracts was conducted using the DPPH method. Individual samples of Jatropha *gossypiifolia* and *Pometia pinnata* extracts were introduced into test tubes containing a DPPH solution. The solution was then incubated for 30 minutes, and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 512 nm.

Preparation of Standard Solutions

Standard solutions were prepared by dissolving ascorbic acid in ethanol solvent, with concentrations ranging from 1 ppm to 10 ppm.

RESULTS AND DISCUSSION

The initial test conducted in this research involved a qualitative phytochemical analysis of the constituents present in each type of sample extract. The identified groups of compounds in the studied extracts included flavonoids, terpenoids, steroids, alkaloids, and tannins (Table 1).

There are reports indicating a relatively high flavonoid content in the leaves of other Jatropha species and the *Pometia pinnata* species (Styani *et al.* 2021; Vega-Ruiz *et al.* 2021) The results from ethanol extracts of *J. gossypifolia* and *Pometia pinnata* leaves are significant, as recent publications highlight the medicinal use of ethanol extracts from these species. Moreover, qualitative phytochemical profiles, similar to those presented in methanol extracts of leaf components from species within the Jatropha genus, have been reported for J. cinerea, J. cordata, and J. curcas (Sulaimon *et al.* 2021; Vega-Ruiz *et al.* 2021).

Several previous studies have demonstrated the antimicrobial activity of plant extracts from *Jatropha gossypifolia* and *Pometia pinnata* (Prastiyanto *et al.* 2021; Rahu *et al.* 2021; Triana *et al.* 2020; Margaretha *et al.* 2020). This antimicrobial activity can be attributed to the presence of specific phytochemical compounds like saponins, tannins, alkaloids, and glycosides. In this study, a combination of ethanol and ethyl acetate extracts from the leaves of *Jatropha gossypifolia* and *Pometia pinnata* exhibited the ability to hinder the growth of the test bacterium *C. acnes.*

From the graph above, it is evident that both ethanol and ethyl acetate extracts exhibited increased antibacterial activity after 48 hours of incubation. The expanded zone of inhibition suggests that the antibacterial effect of the ethanol extract from red *Jatropha gossypifolia* leaves is bactericidal (Brüggemann *et al.* 2021; Rollando 2019). Ethanol

Tabel 1. Phytochemistry assay daun *Jatropha gossypifolia* dan *Pometia pinnata*

Secondary metabolite	***Jatropha gossypifolia* Leaves**		***Pometia pinnata* Stem bark**	
	Ethanol extract	Ethyl acetate extract	Ethanol extract	Ethyl acetate extract
Flavonoid	+	+	+	+
Terpenoid	+	+	+	+
Steroid	+	+	+	+
Alkaloid	+	+	+	+
Tanin	+	+	+	+

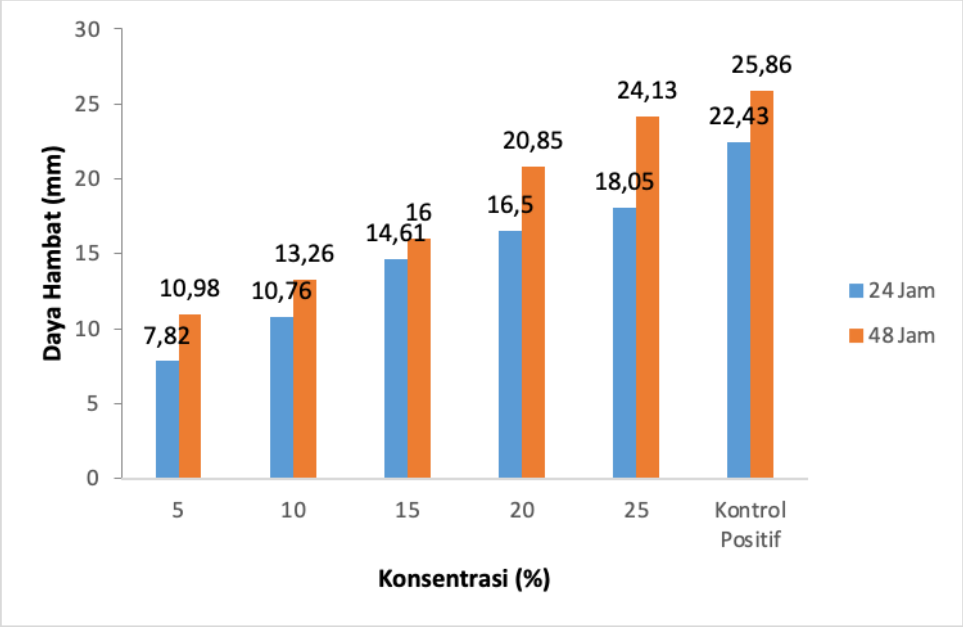

Figure 1. Antibacterial activity of combined ethanol extracts of *Jatropha gossypifolia* and *Pometia pinnata* against *Cutibacterium acnes* with a 1:1 ratio.

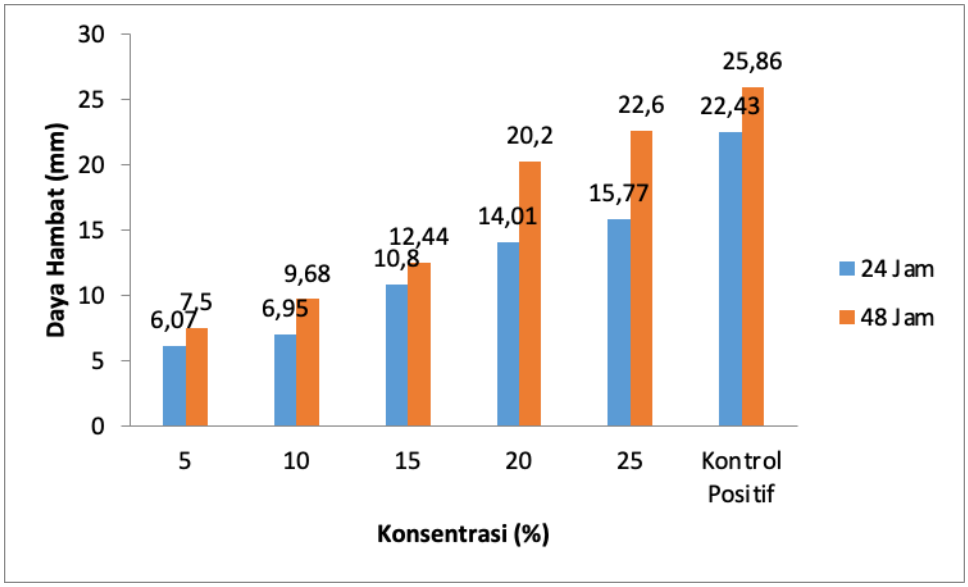

Figure 2. Antibacterial activity of combined ethyl acetate extracts of *Jatropha gossypifolia* and *Pometia pinnata* against *Cutibacterium acnes* with a 1:1 ratio.

extract demonstrated a larger inhibition zone than the ethyl acetate extract. Ethanol extract at a concentration of 25% falls within the strong inhibition category.

Antioxidant activity

The primary source of natural antioxidants is plants, as most of them possess secondary metabolites containing hydroxyl groups within their structure. The primary function of this group is to fortify the defense system against oxidative stress caused by free radicals. Various antioxidant compounds generated by plants stabilize reactive oxygen species (ROS). ROS comprises two categories: free radicals such as hydroxyl and superoxide anion radicals, and non-free radicals, mainly including active oxygen species like singlet oxygen and H_2O_2 (Khan *et al.* 2019). The present test employs DPPH, known for its free radical scavenging activity. Color changes at specific wavelengths arise due to the transfer of hydrogen atoms from antioxidant molecules.

The DPPH method was utilized to ascertain antioxidant capacity, gauged by the extracts' ability to contribute hydrogen and electrons. As indicated in Table 1, the antioxidant capacity of *J. gossypifolia*

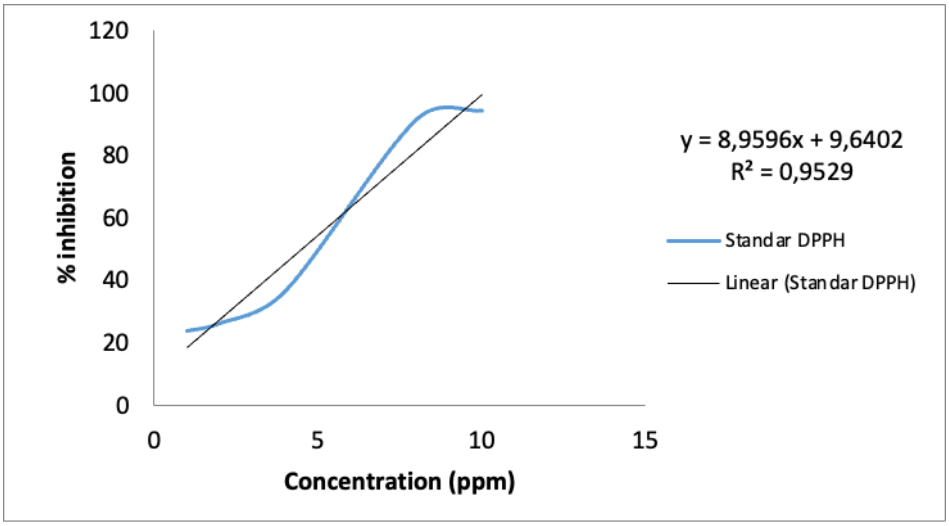

Figure 3. DPPH standard graph using ascorbic acid.

Tabel 2. Results of testing the antioxidant capacity of *Jatropha gossypifolia* leaves and *Pometia pinnata* stem bark

Extract	Capacity (%) AEAC
Ethanol extract *Jatropha gossypifolia* + *Pometia pinnata*	53.23
Ethyl acetate extract *Jatropha gossypifolia* + *Pometia pinnata*	52.96
Ethyl acetate extract *Jatropha gossypifolia*	52.43
Ethyl acetate extract *Pometia pinnata*	53.40
Ethanol extract *Pometia pinnata*	53.18
Ethanol extract *Jatropha gossypifolia*	53.18

leaves (52-53%) closely resembles that of *P. pinnata* leaves (53%). This similarity may stem from a relatively insignificant difference in flavonoid content between both *J. gossypifolia* and *P. pinnata* leaves, as antioxidant activity in plant extracts is often associated with their flavonoid content (Sulaimon *et al.* 2021). Notably, the variation in solvents did not induce a significant alteration in antioxidant capacity. This outcome could be attributed to the presence of flavonoids in both the ethyl acetate and ethanol extracts of leaves from *J. gossypifolia* and *P. pinnata*. Similarly, a blend of ethyl acetate and ethanol extracts from the leaves of *J. gossypifolia* and *P. pinnata* demonstrated only marginal divergence in antioxidant capacity. This observation suggests a potential similarity in flavonoid structure.

CONCLUSION

Jatropha gossypifolia and *Pometia pinnata* stem bark extracts contain flavonoids, steroids, terpenoids, alkaloids and tannins. There is an antibacterial activity of a mixture of ethanol extracts of *J. gossypifolia* and *P. pinnata* leaves with a strong category against *Cutibacterium acnes* acne bacteria at a concentration of 25%. In addition, there was no significant difference in the antioxidant capacity of the mixture of ethanol and ethyl acetate extracts. The antioxidant capacity value is ± 53% AEAC at a concentration of 500 ppm.

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