



## PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATION OF *Pithecellobium jiringa* STEM BARKS EXTRACTS

(Penyaringan Fitokimia dan Antimikrob ke atas Ekstrak Kulit Kayu Batang *Pithecellobium jiringa*)

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### Abstract

*Pithecellobium jiringa* (*P. jiringa*) is economically important due to its various traditional uses in treating hypertension and diabetes (seeds), medicine (leaves) and source of dye for silk (pods). This is a pioneer study that reports its effectiveness as antimicrobial agent from stem bark of *P. jiringa*. The present study was carried out to detect preliminary phytochemicals and antimicrobial of solvent extracts from *P. jiringa* stem bark. Qualitative phytochemical analysis was performed on ethanol and ethyl acetate extracts. These extracts were investigated for their antimicrobial activity of different microorganism tests including bacteria and fungi by using agar disc diffusion technique. Tannins, flavonoids, terpenoids and saponins were the main metabolites found in phytochemical screenings. Both ethanol and ethyl acetate extracts exhibited marked antimicrobial activity against skin disease caused-microbes *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Trychophyton mentagrophytes* with highest inhibition zone (IZ) of 23 mm at 200 mg/mL ethyl acetate extracts. The current study confirmed that the stem bark of *P. jiringa* has a potential source of active antimicrobial agent. Further analysis on the isolation and characterization of compound from *P. jiringa* stem bark, responsible for antimicrobial properties is in progress.

**Keywords:** antifungal activity, antimicrobial activity, inhibition zone, *Pithecellobium jiringa*

### Abstrak

*Pithecellobium jiringa* (*P. jiringa*) adalah penting dari segi ekonomi dengan kegunaan tradisional pelbagai dalam merawat tekanan darah tinggi dan kencing manis (biji), perubatan (daun) dan sumber pewarna untuk sutera (buah). Melalui kajian ini, melaporkan kali pertama, keberkesanannya sebagai agen antimikrob daripada kulit kayu batang *P. jiringa*. Kajian ini telah dijalankan untuk mengesan fitokimia awal dan antimikrob ekstrak pelarut dari kulit kayu batang *P. jiringa*. Analisis fitokimia kualitatif telah dilakukan ke atas ekstrak etanol dan etil asetat. Ekstrak ini telah dikaji untuk aktiviti antimikrob dengan ujian mikroorganisma yang berbeza termasuk bakteria dan kulat menggunakan teknik resapan cakera agar. Tanin, flavonoid, terpenoid dan saponin adalah metabolit utama yang diperolehi dari saringan fitokimia. Kedua-dua etanol dan etil asetat ekstrak mempamerkan tanda aktiviti antimikrob terhadap penyakit kulit yang disebabkan oleh mikrob *Staphylococcus aureus*, *Staphylococcus epidermidis* dan *Trychophyton mentagrophytes* dengan zon perencatan tertinggi (IZ) 23 mm pada 200 mg/mL ekstrak etil asetat. Kajian ini mengesahkan bahawa kulit kayu batang *P. jiringa* mempunyai sumber potensi antimikrob aktif. Analisis lanjut mengenai pengasingan dan pencirian komponen kulit kayu batang *P. jiringa*, bertanggungjawab untuk ciri-ciri antimikrob sedang dijalankan.

**Kata kunci:** aktiviti antikulat, aktiviti antimikrob, zon perencatan, *Pithecellobium jiringa*

### Introduction

*Pithecellobium jiringa* (Jack) Prain is a traditional medicinal plant belongs to the family of Leguminosae which originated from the Southeast Asia [1]. *P. Jiringa* is known as *jering* in Malaysia, *djengkol* in Indonesia and *Luk-nieng* in Thailand. *P. jiringa* is commonly consumed in Malaysia, Indonesia and Thailand. The fruit has been used to eliminate bladder stones when taken as a drink that is mixed with ginger. Traditionally, the plant is also used to treat hypertension and diabetes. The fruit rinds have been identified to be used as ingredients to make traditional soaps, shampoos and detergents. The leaves of *P. jiringa* is used to cure the itchy parts of the human body [2]. In the present day, *P. jiringa* is also being used in the production of organic pesticide as a replacement for synthetic pesticide; since the plant contains djenkolic acid that could kill and inhibit pests. Old folk has also been using its pounded leaves and bark for chest pains, skin ailments, gum pains and toothache [3].

The previous research on the extraction of leaves, pods and seeds of *P. jiringa* using methanol revealed the antimicrobial and antifungal activities against the microorganisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis* from bacteria group while *Microsporum gypsum* from fungi [4]. According to Charungchitraka et al., *Archidendron jiringa* Nielsen lectin had shown low concentration antifungal activity against *Exserohilum turcicum*, *Fusarium oxysporum* and *Colletotrichum cassiicola* while *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans* were observed by minimal inhibitory concentration (MIC) determination [5]. The extraction of *P. Jiringa* seed using methanol had inhibited the bacteria even at low concentration against *Exserohilum turcicum*, *Fusarium oxysporum* and *Colletotrichum cassiicola*. Moreover, the leaves of *P. jiringa* had showed an inhibition zone of fungi that mostly active against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Microsporum gypsum* at 100 mg/mL [1].

Nowadays, the high cost and non-availability of new generation antibiotics with limited effective span have encouraged the increasing in morbidity and death. Thus, a new solution must be produced from other sources with proven antimicrobial properties. As a result, this has led to the new research about the materials of plant origin to find out more effective antimicrobial agents other than to discover the potential useful active ingredients that can be used as source for the synthesis of new antimicrobial drugs and baseline to identify the active compounds that involve in this activity. The scientific value of *P. jiringa* stem bark on the antimicrobial activity under this study can be used to extend its use to the community.

### Materials and Methods

#### Plant extraction

*Pithecellobium jiringa* (*P. jiringa*) stem bark was washed and kept air-dried for one week. The dried plant material was cut into small pieces, and ground twice to produce fine particles. The stem bark of *P. jiringa* was weighed and soaked with ethyl acetate in ratio 1:5 (w/v) for 72 hours. The same stem bark was soaked again with ethanol with same ratio and time of extraction. The mixtures were filtrated and concentrated by using rotary evaporator at 40 °C.

#### Test microorganisms

For antimicrobial and antifungal studies, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Trychophyton mentagrophytes* and *Candida albicans* were obtained from Biological Laboratory 3, Universiti Teknologi MARA (Pahang).

#### Agar preparation

Nutrient agar and Potato dextrose agar (PDA) were used for the microbiological analysis; antimicrobial and antifungal activities of the crude extract in stem bark of *P. jiringa* on the two pure cultures of bacterial and two cultures of dermatophytes. All media were prepared and sterilized on different petri dish that followed the manufacturer's specifications [6].

#### Microbiological assay

The fungal suspension was standardized spectrophotometrically to an absorbance optical density (OD) of 0.1 at 450 nm by using UV-VIS. This adjusted suspension corresponded to  $0.5 - 2.5 \times 10^3$  cells/mL and used as inoculums for antifungal susceptibility testing. Potato Dextrose Agar (PDA) was inoculated with *Trychophyton mentagrophytes* and *Candida albicans* by using cotton buds. Then, all discs with different concentration of ethyl acetate and ethanol

extracts (25, 50, 100 and 200 mg/mL) were aseptically transferred to the inoculated agar plates and later all the plates were incubated for two days at 37 °C [6].

The bacterial suspension was standardized spectrophotometrically to final optical densities (OD) of 0.1 was achieved using UV-VIS at 600 nm. These standardized OD values of inoculums were corresponding to the concentration of  $1.5 \times 10^6$  CFU/mL. Nutrient Agar (NA) was inoculated with *Staphylococcus aureus* and *Staphylococcus epidermidis* by using cotton buds and the plates were allowed to dry for five minutes. Then, all discs with different concentration of extracts (25, 50, 100 and 200 mg/mL) were aseptically transferred to the inoculated agar plates and all the plates were incubated for 24 hours at 37 °C [7].

The activity of microbes was determined by the presence of clear zones of inhibition around the discs. The diameter of the zone of inhibition was recorded in millimetre (mm). Nystatin was used as standard antifungal, ampicillin (10 µg) as standard antibacterial and solvent as a negative control. All determinations were carried out in triplicate [8].

### Phytochemical analysis

Test for alkaloids: 1 mL of each filtrate of extracts was added with a few drops of the Mayer's reagent. The formation of greenish colour or cream precipitate indicates the presence of alkaloids. Test for saponins: 1 mL of each filtrate of extracts was mixed with 10 mL of distilled water. Then, the mixture was shaken vigorously for 30 seconds. The honey comb froth formation indicated the presence of saponins. Test for flavonoids: 1 mL of each filtrate of extracts was mixed with few pieces of magnesium strips and a few drops of concentrated HCl. An orange faint colour of effervescence solution indicated the presence of flavonoids. Test for tannins: 1 mL of each filtrate of extracts was added with three drops of ferric chloride solution. A blue black or blue green precipitate was taken as evidence for the presence of tannins. Test for terpenoids: 1 mL of each filtrate of extracts was added with 2 mL of chloroform. 3 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

### Results and Discussion

Antimicrobial assays of ethanol and ethyl acetate extracts of *P. jiringa* stem bark were screened against bacteria and fungi. Antifungal activity was investigated using two fungal strains *Trichophyton mentagrophytes* and *Candida albicans* as shown in Table 1. The ethanol and ethyl acetate extract showed the maximum diameter of inhibition zone 18.0 and 23.0 mm against *Trichophyton mentagrophytes* at 200 mg/mL and showed no inhibition against *Candida albicans*. The results of both the extracts were correlated with the standard drug thus showed that the ethyl acetate extracts had performed good activity against *Trichophyton mentagrophyte*.

Table 1. Antifungal activity of extracts of *P. jiringa* stem bark on two fungal strains at various concentrations

Fungi	Concentration (mg/mL)	Extracts (Diameter of Inhibition Zone, mm)		
		Ethanol	Ethyl Acetate	Nystatin (Control)
<i>Trychophyton mentagrophytes</i>	25	8.0±0.58	12.0±0.58	12.0±0.1
	50	9.0±1.00	13.0±1.53	
	100	13.0±1.00	19.0±1.53	
	200	18.0±1.00	23.0±1.00	
<i>Candida albicans</i>	25	-	-	30.0±0.05
	50	-	-	
	100	-	-	
	200	-	-	

- sign no inhibition

The antibacterial activity of the ethanol and ethyl acetate extracts of *P. jiringa* stem bark against two standard gram-positive bacterial strains; *Staphylococcus aureus* and *Staphylococcus epidermidis* was investigated. Both of bacteria were the causative bacterium of skin disease in community acquired pneumonia and blood sepsis [9]. The result shown in Table 2 revealed the diameter of zone inhibition was dependent manner with concentration. It means, the higher the concentration of the extract, the larger diameter inhibition zone against microorganisms.

Table 2. Antibacterial activities of extracts of *P. jiringa* stem bark on two strains of bacteria at various concentrations

Bacteria	Concentration (mg/mL)	Extracts (Diameter of Inhibition Zone, mm)		
		Ethanol	Ethyl Acetate	Ampicilin (Control)
<i>Staphylococcus aureus</i>	25	8.0±0.58	7.7±0.00	18.0±0.1
	50	7.5±0.00	8.2±0.58	
	100	8.2±0.53	9.5±0.58	
	200	9.0±1.53	11.7±1.53	
<i>Staphylococcus epidermidis</i>	25	6.0±1.00	7.0±0.00	37.0±0.05
	50	7.0±1.53	12.0±0.58	
	100	9.0±0.58	11.0±1.53	
	200	9.5±0.58	13.0±0.58	

The microbial activity of *Staphylococcus epidermidis* had more efficiently inhibited towards the extracts than *Staphylococcus aureus*. This was revealed by the diameter of zone inhibition for all extracts. At highest concentration (200 mg/mL), 13.0 mm was the largest inhibition zone of ethyl acetate extract was inhibited against *Staphylococcus epidermidis* compared to *Staphylococcus aureus* with 11.7 mm of zone inhibition. For the control activity, all solvents extract used as the negative control did not inhibit the bacteria. Meanwhile, the positive control, Ampicilin had strongly inhibited both bacteria; *Staphylococcus aureus* with 18.0 mm inhibition zone and *Staphylococcus epidermidis*, 37.0 mm inhibition zone. This means the extracts were active towards bacteria.

The phytochemical screening of the ethyl acetate and ethanol extracts studies on the stem bark of *P. jiringa* revealed that both crude extracts were rich in saponins, flavonoids, tannins and terpenoids but alkaloids does not present as shown in Table 3. These compounds have been known to be biologically active and therefore aid the antimicrobial activities of *P. jiringa* stem bark.

Table 3. Phytochemical screening test for *P. jiringa* stem bark extracts

	Alkaloids	Saponins	Flavonoids	Tannins	Terpenoids
Ethyl acetate	-	++	+	++	++
Ethanol	-	++	+	++	++

++: strongly presence, +: presence, -: absence

Saponins were reported as a major components acting as antifungal secondary metabolite [10, 11]. Therefore, saponins that found in stem bark of *P. jiringa* extracts have supported the usefulness of this plant in managing inflammation. Parekh and Chanda were reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues [12]. The presence of tannins in stem bark of *P. jiringa* shows its potential for treatment of different ailments. Flavonoids in *P. jiringa* stem bark extracts can exhibit a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic,

analgesic, anti-allergic, cytostatic and antioxidant properties [13]. Study done by Quinlan et al. revealed that some medicinal plants which exhibited antibacterial activities contained terpenoids compound [14]. Terpenoids are also responsible for the diverse pharmacological actions of the crude extract used.

### Conclusion

Stem bark of *P. jiringa* contains important secondary metabolites (tannins, flavonoids, terpenoids, and saponins) thus has proven the therapeutic potentials of the plant. The screening of ethyl acetate crude extract of *P. jiringa* stem bark showed active activities against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Trychophyton mentagrophytes*. The largest diameter of inhibition zone for antibacterial is 13 mm which is moderately inhibited while for antifungal is 23 mm which indicates strongly inhibited. Therefore, it can be concluded that *P. jiringa* stem bark can be a potential source of active antimicrobial agents, and a detailed assessment of its in-vivo potencies and toxicological profile should be performed. This plant also appears to be the clinical, scientific, and pharmacologic basis for the use of herbal preparations. Further study on isolation and characterization of the active compounds from the crude *P. jiringa* stem bark extract is important for drug synthesis.

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