A review on the bioactivities of Justicia gendarussa

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Abstract. Willow-leaved justicia (Justicia gendarussa) can be found wild or cultivated in Indonesia, India, China, Malaysia, Sri Lanka, the Philippines, and Bangladesh. In Indonesia, willow-leaved justicia leaves are widely used in traditional medicines by Indonesian local inhabitants. The present work provides a review addressing bioactivities of willow-leaved justicia. The observed bioactivities of willow-leaved justicia include antioxidant, hepatoprotective, anti-inflammation, anti-microbial (anti-fungal, anti-bacterial, anti-viral), anthelmintic, anti-cancer, anti-sickling, larvicidal and adulticidal activities.

1. Introduction
Willow-leaved justicia (Justicia gendarussa) is a dicotyledonous plant with a bush-like appearance that grows to a maximum height of 1.5 meters. This plant has inflorescence on spikes of 10 cm long and 1.5 cm wide with 1.6-2 cm long subsessile flowers white with purple lines and spots in them, leaves are white, green, and gray with lanceolate-shaped and hairy on both sides with a length of 7–14 cm and a width of 1–2.5 cm, and stem-shaped and hairy fruits with a length of 12 mm [1].

Willow-leaved justicia can be found wild or cultivated in Indonesia, India, China, Malaysia, Sri Lanka, the Philippines, and Bangladesh. A wide variety of biologically active constituents such as flavonoids, alkaloids, steroids, terpenoids, saponins, phenolic compounds and carbohydrates are present in this plant. The leaves also contain friedelin, lupeol, ß-lsitosterol and aromatic amines [2]. The leaves of this plant have been proven to have anti-angiogenic activity [3], antioxidants [4], anti-bacterial[5], anti-fungal [6], anti-rheumatism [7], and anti-inflammatory [8]. In addition, aerial parts of plants showed in vitro type 1 reverse transcriptase HIV inhibitor activity [9], and anti-inflammatory [10]. This plant has also been used by people in Papua as male contraception [11].

Figure 1. Bioactivities in willow-leaved justicia
2. Antioxidant

Several studies have proven the antioxidant activity found in the willow-leaved justicia plant. Flavonoid compounds have been shown to play a role in antioxidant activity (Table 1) possessed by ethanol extracts of willow-leaved justicia leaves [4].

| Antioxidant                                  | IC50 value (µg/ml) |
|----------------------------------------------|--------------------|
| DPPH radical scavenging                      | 123.09 ± 3.01      |
| NO scavenging                                | 643.00 ± 61.10     |
| β-CLAMPS                                     | 132.30 ± 6.03      |
| OH radical scavenging                        | 68.50 ± 11.50      |
| Anti-lipid peroxidation activity             | 68.13 ± 1.38       |

Source: Mruthunjaya & Hukkeri [4]

In addition, other studies have also proven the presence of antioxidant activity and hepatoprotective activity in the methanol extract of willow-leaved justicia leaves [12]. The results of this study showed that weak antioxidant activities were found in the methanol extract (IC50 = 222.68 µg/ml) and water extract (IC50 = 455 µg/ml) of willow-leaved justicia leaves, compared to the control, such as ascorbic acid (IC50 = 4.17 µg/ml), gallic acid (IC50 = 1.86 µg/ml), and BHT (IC50 = 29.08 µg/ml).

3. Hepatoprotective activity

Hepatoprotective activity is the ability of test sample to protect the liver against toxicant. Hepatoprotective activity test of willow-leaved justicia was carried out in albino Wistar rats induced with CCl4 hepatotoxin and silymarin compounds as controls [12]. The results of the study proved that the methanol extract of willow-leaved justicia leaves had hepatoprotective activity against rat samples with the most effective dose of 300 mg/kg that able to reduce the SGOT, SGPT, total bilirubin, direct bilirubin, and SALP from 1422.33 to 591.00 IU/L, 930.33 to 644.66 IU/L, 1.94 to 0.51 mg/dL, 0.41 to 0.28 mg/dL, 858.16 to 551.16 IU/L, respectively, and are comparable to control drug silymarin at dose 100 mg/kg (SGOT = 574.33 IU/L; SGPT = 440.66 IU/L; total bilirubin = 0.55 mg/dL; direct bilirubin = 0.21 mg/dL; SALP = 546.83 IU/L). However, further research is needed to determine which pure compounds are responsible for this activity.

4. Anti-inflammation

Anti-inflammatory activity was found in both types of research models on rheumatic rats, which are Freund’s Complete Adjuvant (FCA) and Collagen Induced Arthritis (CIA) as shown in Table 2[7]. The results showed that the ethanol extract of willow-leaved justicia leaves was able to inhibit mouse paw edema by 43% in the FCA model and 47% in the collagen model, whereas positive control of aspirin only inhibited paw edema by 26% in the FCA model and 38% in the collagen model. Based on this research, the ethanol extract of willow-leaved justicia leaves has been shown to have arthritis anti-inflammatory activity tested on mice because it has determinant parameter values that are comparable to normal mice (group I). As seen in Table 2 that both research models proved an increasing amount of Hb and RBC and decreasing amount of WBC, ESR, CRP, and copper from group II rheumatic mice into group IV rheumatic mice treated with ethanol extract of willow-leaved justicia leaves.

Anti-inflammatory activity has also been demonstrated in other studies with edema models induced by carrageenan and formalin [8]. In the carrageenan induction model (CG), mousepaw edema was successfully inhibited by 56.92% by plant extracts in a dose of 125 mg/kg, by 61.53% in a dose of 250 mg/kg extract, and by 75.38% in a dose 500 mg/kg. Whereas in the formalin induction (FN) model, mousepaw edema was successfully inhibited by 55.55% in a dose of 125 mg/kg, 70.44% in a dose of 250 mg/kg extract, and 74.22% in a dose of 500 mg/kg. These results showed the anti-inflammatory activity in the ethanol extract of willow-leaved justicia leaves is quite effective because the results were comparable to control (10 mg/kg indomethacin), which is 84.61% inhibition in the CG model and 86.66% in the FN model.
In addition, anti-inflammatory activity has also been found in the ethanol extract of aerial parts of willow-leaved justicia plants through research with edema models induced by carrageenan [10]. In carrageenan induction model, paw edema in mice was successfully inhibited by 21.42% by plant extracts in a dose of 250 mg/kg and by 32.95% in a dose of 500 mg/kg. These results proved the anti-inflammatory activity in the ethanol extract of willow-leaved justicia leaves is quite effective because the results were comparable to control (300 mg/kg aspirin) which can inhibit edema by 66.79%.

### Table 2. Anti-inflammatory activities of willow-leaved justicia leaves extracts

| Induced or parameters | I* | II* | III* | IV* |
|-----------------------|----|-----|------|-----|
| **Hb (g/dL)**         | 12.25 | 9.00 | 9.67 | 11.75 |
| RBC (×10^6/mm³)       | 4.48 | 3.76 | 3.78 | 4.14 |
| WBC (×10^3/mm³)       | 7.34 | 17.43 | 12.78 | 8.88 |
| ESR                   | 3.33 | 10.67 | 10.50 | 5.17 |
| CRP (µg/ml)           | 172.9 | 425.7 | 254.2 | 285.0 |
| Copper (µg/ml)        | 103.2 | 186.1 | 138.1 | 124.3 |

**FCA-induced**

**Collagen-induced**

*I = Group of normal mice induced with gum acacia
*II = Group of rheumatic mice induced with FCA/collagen
*III = Group of rheumatic mice induced with FCA/collagen and aspirin (control)
*IV = Group of rheumatic mice induced with FCA/collagen and ethanol extract of willow-leaved justicia leaves

**Source:** Paval et al. [7]

Anti-inflammatory activity has also been tested using the HRBC (Human Red Blood Cells) membrane stabilization method [13]. Among the five types of extracts tested, hexane, diethyl ether, dichloromethane, ethyl acetate, and methanol, methanol extract of willow-leaved justicia leaf which has the strongest anti-inflammatory activity in inhibiting the release of prostaglandins or other inflammatory mediators from cell membranes by stabilizing the membrane because it shows the most inhibitory percentage close to control (sodium diclofenac) (Table 3).

### Table 3. Anti-inflammatory activities of willow-leaved justicia leaves extracts

| Treatment        | Concentration (mg/ml) | Haemolysis (%) | Inhibition (%) |
|------------------|-----------------------|----------------|----------------|
| Control          |                       | 0.345          |                |
| Hexane           | 250-1000              | 42.90-46.96    | 53.03-57.10    |
| Diethyl Ether    | 250-1000              | 39.13-45.22    | 54.78-60.87    |
| Dichloromethane  | 250-1000              | 33.91-37.97    | 62.03-66.09    |
| Ethyl Acetate    | 250-1000              | 28.12-38.26    | 61.74-71.88    |
| Methanol         | 250-1000              | 23.80-27.54    | 72.46-76.20    |
| Positive Control | Sodium diclofenac     | 50             | 25.85          | 74.14          |

**Source:** Nirmalraj, Ravikumar, Mahendrakumar, Bharath, & Perinbam [13]

5. Anti-microbial: antifungal, antibacterial, and anti-viral activities

Many studies have proven the existence of antimicrobial activity in plants of *Justicia gendarussa*, such as antibacterial, antifungal, anthelmintic, and antiviral. The antibacterial activity of this plant has been tested for its strength from several types of extracts against the bacteria *Staphylococcus aureus, Streptococcus mutans, Bacillus substilis, Micrococcus luteus, Proteus vulgaris, Klebsiella...*
pneumoniae, Escherichia coli, and Shigella flexneri. Among the hexane, diethyl ether, dichloromethane, ethyl acetate, and methanol extracts tested, the methanol extract of willow-leaved justicia leaves had the strongest antibacterial activity against all 8 types of bacteria that is seen from the value of the zone of inhibition that is ranging from 7-12 mm. Phytochemical compounds such as flavonoids, saponins, tannins, terpenoids, O-disubstituted aromatic amines, 2-aminobenzyl alcohols, O-methyl ethers, friedelin, lupeol, and β-cystosterol, are believed to be responsible for the presence of antibacterial activity in willow-leaved justicia[13].

According to the research of Sharma, Saikia, Kotoky, Kalita, & Devi [6], young leaf and shoot extracts from the willow-leaved justicia plant have antifungal activity against fungi causing dermatophytes. Among the three extracts tested, chloroform extract showed the strongest activity against Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum gypseum, and Microsporumffulvum with inhibition zone ranging from 9-13 mm at 50 mg/ml and 19-22 at 100 mg/ml. However, further research is needed to find out the compounds responsible for this activity.

Research by Woradulayapinij, Soonthorncharennon, & Wiwat[9] has shown that some tropical plants can help cure HIV/AIDS. The results of this study prove the presence of reverse transcriptase HIV-1 inhibitory activity in ethanol and water extracts from willow-leaved justicia plant parts which are exposed to air. Anti-HIV activity in ethanol extract of willow-leaved justicia plants is relatively weak with IR value of 16.82% at a concentration of 200 µg/ml whereas activity in water extract is classified as very strong with IR value of 90.75% at the same concentration. The difference between the two inhibitory values is very far, so it is suspected that the components responsible for this activity are polar and water-soluble compounds. Further research then stated that protein is an active component that has anti-HIV-RT activity in this plant. The most recent research found that the powerful compound isolated from willow-leaved justicia that possess anti-HIV activity is patentiflorin A [14].

6. Anthelmintic activity

In addition, antiparasitic activity in anthelmintic is also found in this plant extract. Previously it was known that earthworms are very susceptible to willow-leaved justicia. Therefore, this activity was tested in vitro on earthworms Pheretimaphostuma[15]. The results showed that the methanol extract of leaves and stems of willow-leaved justicia at a concentration of 50 mg/ml was able to immobilize the worm at minute 35 and 41 and was able to kill the worm at minutes 70 and 89, while the drug albendazole in the concentration of 10 mg/ml could immobilize the worm at minute 17 and deadly at minute 48. This proves that anthelmintic activity in the extracts of the leaves and stems of willow-leaved justicia exists but is not as effective as the albendazole worming drug. The methanol extract of this plant contains lupeol, stigmasterol, and 16-hydroxylupeol which are thought to play a role in the existence of this anthelmintic activity. However, further research is needed to find out the compounds responsible for anthelmintic activity and to test their effectiveness in vivo.

7. Anticarcinogenic and antiangiogenic activities

Research by Ayob, Samad, & Bohari[16] proved that plants of willow-leaved justicia, both methanol extracts and pure compounds resulting from their isolation, have activities against cancer cells HT-29, HeLa, and BxPC-3. The leaf extract of the J. gendarussa plant from the Mersing region shows the strongest level of toxicity to these cancer cells. Whereas leaf extracts obtained from other regions, such as Muar, Skudai, Batu Pahat, and Pulai, showed a moderate level of toxicity and some were even considered weak.

Meanwhile, pure compounds that were successfully isolated from these plants, which arecaempherol and naringenin, showed a very strong level of toxicity to the three cancer cells tested with IC₅₀ values ranging from 5–23 µg/ml (Table 4). This proves that flavonoid derivatives are compounds that play a role in the anticancer activity possessed by this plant.In addition, Periyanayagam, Umamaheswari, Suseela, Padmini, & Ismail [3] proved that water and ethanol extracts from the J. gendarussa plant have in vitro antiangiogenic activity using formation of a chorioallantoicmembranemethod (Table 5) and state that this extract is safe and has the potential to
replace drugs, but further research is needed regarding the pure compounds responsible and their *in vivo* effectiveness.

### Table 4. Cytotoxicity IC$_{50}$ value of willow-leaved justicia against cancer cells

| Methanol extract of *Justicia gendarussa* | IC$_{50}$ (μg/ml) |
|------------------------------------------|-------------------|
| Mersing                                  | HT-29 21, HeLa 22, BxPC-3 16, CHO (control) 28 |
| Muar                                     | 65, 88, 157, 108 |
| Skudai                                   | 76, 39, 105, 88 |
| Batu Pahat                               | 36, 146, 410, 190 |
| Pulai                                    | 39, 205, 264, 305 |

| Compounds | IC$_{50}$ (μg/ml) |
|-----------|-------------------|
| Kaemferol | 6, 5, 23, 14 |
| Naringenin| 19, 15, 57, 21 |

**Source:** Ayob, Samad, & Bohari [16]

### Table 5. Antiangiogenic activity of willow-leaved justicia plant extracts

| Inhibition* |
|-------------|
| Water extract |
| 25 µg/pellet | 0.70 ± 0.20 |
| 50 µg/pellet | 0.90 ± 0.10 |
| 100 µg/pellet| 1.00 ± 0.20 |
| Ethanol extract |
| 10 µg/pellet | 0.40 ± 0.10 |
| 25 µg/pellet | 1.00 ± 0.20 |
| 50 µg/pellet | 1.30 ± 0.10 |
| Control |
| β, 1, 4 galactan sulphate 50 | 1.20 ± 0.20 |

*0* = no or weak effect  
*1* = medium effect  
*2* = strong effect

**Source:** Periyanayagam, Umamaheswari, Suseela, Padmini, & Ismail [3]

8. **Anti-sickling activity**  
Mpiana et al. [17] conducted research on anti-sickling activity in *J. gendarussa* plants to replace drugs or therapies that are classified as dangerous and expensive to cure sickle cell anemia (SS). This study found that anthocyanin compounds play a role in anti-sickling activity. The leaves of the willow-leaved justicia plant gave ED$_{50}$, NRmax, and MCN values from anthocyanin of 0.44 µg/ml, 87.1%, and 7.6 µg/ml. This anthocyanin causes hemolysis of drepanocytes shown from the absorbance value which decreased by 28% from 1.80 to 1.30 in 60 minutes. These results indicate that anthocyanins from this plant have antihemolytic activity in SS erythrocytes.

9. **Larvicidal and adulticidal activities**  
In addition to the activities previously described, the *J. gendarussa* plant has been shown to have other unique activities, which is larvicidal and adulticidal activities. This activity demonstrates the ability to kill larvae and adult mosquitoes. Senthilkumar, Varma, and Gurushubramanian [18] research tested the larvicidal and adulticidal activity of the leaves of willow-leaved justicia plant against the main mosquitoes that cause malaria, *Anopheles stephensi*. The ethanol extract of willow-leaved justicia leaves has LC$_{50}$ and LC$_{90}$ values of 4.160 ppm and 279.748 ppm against III instar larvae of *A. stephensi*. Meanwhile, ethanol extracts of willow-leaved justicia leaf had LC$_{50}$ and LC$_{90}$ values of 0.668 ppm and 16.328 ppm against *A. stephensi* adult mosquitoes. In addition, the effect of willow-leaved justicia plant extracts on the life cycle of *A. stephensi* after treatment was determined from
larvar mortality values of 55.6%, puspal mortality of 19.3%, and malformed adults by 25.1%. These results indicate the possibility of willow-leaved justicia plants can be used as medicine to control mosquitoes at a low price.

References
[1] Singapore Government Agency 2019 Justicia gendarussa 'Variegata' Online: https://www.nparks.gov.sg/florafaunaweb/flora/2/1/2160
[2] Kavitha K, Sridevisangeetha K S, Sujatha K and Ummaheswari S 2014 J. Pharm. Res. 8 990-7
[3] Periyanayagam K, Umamaheswari B, Suseela L, Padmini M and Ismail M 2009 Am. J. Infect. Dis. 5 180-2
[4] Mruthunjaya K and Hukkeri V I 2007 Nat. Prod. Sci. 13 199-206
[5] Sivasakthi A and Vijayalakshmi M 2014 Int. J. Ethnomed. Pharmacol. Res. 2 44-50
[6] Sharma K K, Saikia R, Kotoky J, Kalita J C and Devi R 2011 Int. J. PharmTech. Res. 3 1635-1640
[7] Paval J, Kaitheri S K, Potu B K, Govindan S, Kumar R S, Narayanan S N and Moorthoth S 2009 Clinics (Sao Paulo)64 357-60
[8] Shikha P, Latha P G, Suja S R, Anuja G I, Shyamal S, Shine V J, Sini S, Kumar N M and Rajasekaran S 2010 Indian J. Nat. Prod. Resour. 4 156-61
[9] Woradulayapinij W, Soonthornchareonnon N and Wiwat C 2005 J. Ethnopharmacol. 101 84-9
[10] Jothimanivannan C, Kumar R S and Subramanian N 2010 Int. J. Pharmacol. 6 278-83
[11] Moeso M and Agus P 1985 Laporan Perjalanan ke Jayapura Sentani, Irian Jaya (Yogyakarta: Gadjah Mada University)
[12] Krishna K L, Mruthunjaya K and Patel J A 2009 Int. J. Biol. Chem. 3 99-110
[13] Nirmalraj S, Ravikumar M, Mahendrakumar M, Bharath B and Perinbam K 2015 J. Plant Sci. 10 1-5
[14] Zhang H, Rumschlag-Booms E, Guan Y, Wang D, Liu K, Li W, Nguyen V H, Cuong N M, Soejarto D D, Fong H H S and Rong L 2017 J. Nat. Prod. 80 1798-1807
[15] Saha M R, Debnath P C, Rahman M A and Islam M N U I 2012 Bangladesh J. Pharmacol. 7 50-3
[16] Ayob Z, Samad A A and Bohari S P M 2013 JurnalTeknologi 64 45-52
[17] Mpiana P T, Bokota M T, Ndjele M B L, Mudogo V, Tshibangu D S T, Ngabolua K N, Atibu E K, Kwembe J T K and Makelele L K 2010 Int. J. Biol. Chem. Sci. 4 1953-61
[18] Senthilkumar N, Varma P and Gurusubramanian G 2009 Parasitol. Res. 104 237-44