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Orok-Orok (Crotalaria pallida Aiton): Botany, Use and Toxicity

Marina Silalahi

Department of Biology education, Faculty of Teacher Training and Education, Universitas Kristen Indonesia, Cawang, Jakarta Timur 13510

E-mail: marina_biouki@yahoo; marina.silalahi@uki.ac.id

Abstract—Crotalaria pallida (Fabaceae) is a plant introduced in Indonesia and has been long used as a traditional medicine. The aim of this study was to explain the botany, bioactivity and toxicity of C. pallida. The research method is through reviewing literature published online, using the keywords C. pallida and bioactivities of C. pallida. Data were analyzed qualitatively to explain botanical information, bioactivity and toxicity of C. pallida. The characteristic C. pallida is trifoliolatus leaves, yellow compound flowers and green young pods. The bioactivity C. pallida is anti-inflammatory, anti-microbial, antioxidant, estrogenic, inhibits tyrosinase, anti-diabetes mellitus, and anti-cancer. The seeds and flowers of C. pallida has dehydropyrrolizidine alkaloids which are hepatotoxic and genotoxic and cause acute poisoning to animals and humans. The bioactivity of C. pallida to inhibit the tyrosinase enzyme has great potential to be developed as a natural skin whitener, but further research needs to be done.

Keywords— Crotalaria pallida, tyrosinase, alkaloid dehydropyrrolizidine

I. INTRODUCTION

Orok-orok or *Crotalaria pallida* (Fabaceae) is an introduced plant in Indonesia (Powo 2023), but it is easily found in neglected land or roadsides as a weed and it is also used to fertilize the soil (Savaris et al 2019). This plant is easily recognized by the yellow flower clusters that appear at the ends of the branches or stems. The *C. pallida* has been recorded in the traditional Indian medicine system, namely Ayurveda (Bolleddu et al 2022).

Researchers report that *C. pallida* has long been used as an ingredient in traditional medicine. Local people in India consume a decoction made from young leaves of *C. pallida* in the morning for the treatment of various diseases such as anthelmintic, fever and wound healing (Panda et al 2015b). The local people in South Sulawesi, *C. pallida* is empirically used to treat cancer (Rumondor et al 2017). Pharmacological studies of *C. pallida* have anti-inflammatory, antimicrobial and antifungal activities (Boldrin et al 2013). Ethanol, ethyl acetate, chloroform, petroleum ether and water extracts of *C. pallida* have different antimicrobial, antioxidant and anti-inflammatory, lipoxygenase, xanthine oxidase (XO), acetylcholinesterase activities (Govindappa et al 2011; Govindappa et al 2011b). The leaves of *C. pallida* have significant anti-nociceptive and anti-inflammatory effects, confirming their traditional use in traditional medicine related to healing pain and inflammation (Bulbul et al 2017).

Although *C. pallida* is widely used as a traditional medicine, on the other hand, researchers have also reported its toxic effects, such as Savaris et al (2019), Diaz et al (2003), Gomes et al (2005), Diaz et al (2014), and Prada et al (2020). The seeds and flowers of *C. pallida* contain pyrrolizidine alkaloids (PA) (Savaris et al 2019), namely a type of retronecine cyclic diester monocrotaline, crotaleschenine, integerrimine, usaramine, and its N-oxide is a serious health threat to humans and livestock

Corresponding Author: Marina Silalahi

(Prada et al 2020). However, Ukil et al (2017) stated that *C. pallida* seeds are a non-traditional source of protein (22%) but their role as an important source of protein has not been explored (Ukil et al 2017).

Plants used by humans as traditional medicine sometimes have toxic effects, therefore comprehensive information is needed so that negative impacts can be avoided. The studies comprehensive of *C. pallida* are still limited. The purpose of writing this article is to explain botany, bioactivity and toxicity of *C. pallida*.

II. Methods

This study is based on literature published online, especially on Google Scholar. Keywords used in searching articles include *C. pallida*, and bioactivities of *C. pallida*. The data obtained were analyzed qualitatively to obtain in-depth information regarding the botany, bioactivity and toxicity of *C. pallida*. The articles we use are articles related to the botany, secondary metabolites and bioactivity of *C. pallida*. To complete the description, we carried out an exploration of the surrounding environment.

III. Results and Discussion

A. Botany of *Crotalaria pallida* Aiton

Fabaceae is one of the large families of the Magnoliopsida class which has around 440 genera and around 12,000 species (Rodríguez-Riaño et al 1999). The *Crotalaria* genus has around 600 (Mosjidis & Wang 2011) – 700 species (Maberley 2008) which are mostly distributed in tropical and subtropical areas. This genus belongs to the subfamily Papilionoideae (Mosjidis & Wang 2011). Crotalaria contains pyrrolizidine alkaloids, which ingested in sufficient quantities can be toxic to animals and birds (Mosjidis & Wang 2011).

Description: *C. pallida* has an upright shrub habit with a height of 1–2 m and has many branches. Compound leaves with 3 leaflets; Leaf stalk 4cm long. The leaves are oval in shape with a size of 3–6 x 2–3 cm, and the apex is blunt – emarginate with a pointed base and has a membrane. The leaf stalks are 3mm long and hairy. Flowers appear at the ends (terminal) of branches or stems in an arrangement of racemes measuring up to 25cm. Flower stalk 5cm long; linear bracts 4mm long, caducous; subulate bracteoles, 1–2 mm long. Calyx tube campanulate 3mm long, lobes lanceolate, 4-5 mm long, pointed at apex, adpressed pubescent. Corollas yellow with purple stripes, twice as long as petals; vexiclum yellowish purple, oval-elliptical, ca. 1.2 x 0.8 cm, blunt-rounded at apex, claws ca. length 3mm; elliptical calyx wings, ca. 1.0 x 0.3 cm; crescent keel petals, ca. 1.2 x 0.5 cm, ciliated on both edges. The body resistance of the sarong is ca. length 8mm; free filament 4–8 mm long; linear-lanceolate anthers, ca. length 3mm; style ca. 1 cm long, curved, pubescent on inner side. Pods oval or subcylindrical, 3–4 x 0.6–0.7 cm, short stipitate, sometimes slightly curved, glabrescent. Seeds 40–60, reniform, ca. 3 x 2 mm, brown (Gambar (Patil et al 2014).

Figure 1. *Orok-orok or Crotalaria pallida* Aiton. A. Habitus showing trifoliolatus leaves; B. Flowering; C. Flowers are blooming; D. Young pods.

B. Uses and Bioactivities of *Crotalaria pallida* Aiton

Crotalaria pallida has long been used as a traditional medicine to treat various diseases. The use of plants as traditional

medicine is related to their bioactivity and secondary metabolite content. The following will further explain the bioactivity of *C. pallida* as anti-inflammatory, anti-microbial, antioxidant, estrogenic, inhibiting tyrosinase, anti-diabetes mellitus, and anti-cancer.

1. Anti Inflammations

Inflammation is one part of the body's defense mechanism and plays a role in the healing process which provides analgesic and antipyretic effects so that it can reduce pain. Aspirin is used as a standard drug for anti-inflammatory activity studies (Govindappa et al 2011). Compounds used to treat inflammation are called anti-inflammatory (Bulbul et al 2017). In vitro anti-inflammatory activity can be evaluated using albumin denaturation, membrane stabilization and proteinase inhibitory activity (Govindappa et al 2011).

Traditionally *C. pallida* is used to treat urinary disorders, external application as a poultice to treat pain, joint swelling and reduce fever (Bulbul et al 2017). The bioactivity of *C. pallida* as an anti-inflammatory has been reported by Weng et al (2003), Ko et al (2004), Govindappa et al (2011), and Bulbul et al (2017). The *C. pallida* extract has antinociceptive effects (the ability to reduce sensitivity to stimuli that cause pain) in mice (Bulbul et al 2017). The ethanol extract of *C. pallida* leaves has anti-inflammatory properties (Bulbul et al 2017; Govindappa et al 2011) and inhibits the activity of xanthine oxidase, acetylcholinesterase, and anti-lipoxygenase enzymes (Govindappa et al 2011). In the writhing test on mice induced by acetic acid then *C. pallida* extract at doses (50 mg/kg and 100 mg/kg bw) significantly reduced pain by 37.50% and 47.70% respectively (Bulbul et al 2017).

The *C. pallida* bioactivity as an anti-inflammatory is related to its bioactive compound content. Ethanol, ethyl acetate and petroleum ether *C. pallida* extracts contain phenols, alkaloids, terpenoids, saponins, phenols, steroids and tannins (Govindappa et al 2011). Flavonoids are chemical mediators that are suppressed in inflammatory cells so they have value in the treatment and prevention of central and peripheral diseases associated with excess production of chemical mediators (Ko et al 2004). The compounds apigenin and 2′-hydroxygenistein, isolated from *C. pallida* bark showed an inhibitory effect on the release of β-glucuronidase and lysozyme from rat neutrophils in response to formyl-Met-Leu-Phe/cytochalasin B (fMLP/CB). The compounds daidzein and 2′-hydroxydaidzein inhibited the release of lysozyme and β-glucuronidase from rat neutrophils in response to fMLP/CB with IC50 values of 26.3 ± 5.5 and 13.7 ± 2.6 μM, respectively (Ko et al 2004).

Compounds crotafurans A and B of *C. pallida* bark inhibited lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage-like cells' Nitric Oxide (NO) production significantly (Weng et al 2003). The crotafurans B compound also inhibited NO production stimulated by LPS/interferon-γ (IFN-γ) in N9 microglial cells with an IC50 value of 9.4 ± 0.9 μM. Crotafurans A compound produced a concentration-dependent inhibition of the release of β-glucuronidase and lysozyme from rat neutrophils in response to formyl-Met-Leu-Phe/cytochalasin B (fMLP/CB) with IC50 values of 7.8 ± 1.4 and 9.5 ± 2.1 μM, respectively (Weng et al 2003).

Apigenin and daidzein compounds showed significant concentration-dependent inhibitory effects on superoxide anion formation in rat neutrophils stimulated with fMLP/CB with IC50 values of 3.4±0.3 and 25.1±5.0 μM, respectively (Ko et al 2004). The apigenin compound showed inhibition of NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages and LPS/interferon-γ-stimulated N9 microglial cells (IFN-γ) (Ko et al 2004).

2. Anti-Microbial

Microbial resistance to antibiotics has led to the search for more effective antimicrobial agents among plant materials that can serve as sources and templates for the synthesis of new antimicrobial drugs (Ukil et al 2016). Pharmacological research on the effectiveness of *C. pallida* inhibits the growth of Gram-positive and Gram-negative bacteria (Ukil et al 2019). The C. pallida inhibits the growth of bacteria such as *Escherichia coli* (Islam et al 2018; Ukil et al 2016; Kiruthiga et al 2014), *Staphylococcus aureus, Pseudomonas aeruginosa* (Islam et al 2018; Kiruthiga et al 2014), *Bacillus cereus* (Islam et al 2018), *Bacillus subtilis, Acinetobacter junii* (Ukil et al 2016), *Klebsilla pneumonieae, Bacillus* sp. (Kiruthiga et al 2014), *Vibrio cholera, Shigella flexneri,* and *Shigella dysenteriae* (Alam et al 2014).

The activity of *C. pallida* as an antibacterial varies greatly, which is influenced by various factors including the substance used for extraction, type of bacteria and concentration. Essential oil from *C. pallida* has antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Acinetobacter junii*) (Ukil et al 2016). Petroleum ether

and chloroform extracts of *C. pallida* stem showed mild to moderate antibacterial activity (Islam et al 2018). The ethanol extract of *C. pallida* leaves showed antibacterial activity against the bacterial strains such as *Vibrio cholera, Shigella flexneri, Shigella dysenteriae*, compared to the standard drug kanamycin (30 µg/disk) (Alam et al 2014). At a concentration of 400 mg/ml, *C. pallida* root ethanol extract showed activity similar to chloramphenicol, a standard antibiotic (Kwaji et al 2013).

3. Antioxidants

Antioxidants play an important role in protecting against cell damage by reactive oxygen species (Islam et al 2018; Govindappa et al 2011b). Plant phenolic compounds have strong antioxidant properties (Govindappa et al 2011b). The bioactivity of *C. pallida* as an antioxidant have been reported by Islam et al (2018); Govindappa et al (2011b), Shen et al (2022), Umashankar et al (2012), and Alam et al (2014).

Antioxidant activity can be measured through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test and the ferric reducing antioxidant power (FRAP) (Govindappa et al 2011). Ascorbic acid and butylated hydroxytoluene (BHT) were used as standards for antioxidant activity (Govindappa et al 2011). Compounds isolated from 95% ethanol extract of *C. pallida* seeds had moderate antioxidant activity (Shen et al 2022). Petroleum ether, chloroform, methanol and water extracts of *C. pallida* stem showed remarkable antioxidant activity (Islam et al 2018).

Ethanol, ethyl acetate and petroleum ether extracts have strong scavenging activity in both the DPPH and FRAP methods. Ethanol, ethyl acetate and petroleum ether have shown free radical inhibition of 88, 72 and 73 and 3617.89 ± 0.03, 2189.33 ± 0.03 and 1133.26 ± 0.01, respectively (Govindappa et al 2011). The *C. pallida* stem petroleum ether extract showed DPPH radical scavenging activity with an IC50 value of 126.96 µg/ml (Islam et al 2018).

4. Estrogenic

Estrogenic compounds are compounds that are related to the presence of the hormone estrogen (a group of hormones that play an important role in the development and growth of women's sexual characteristics and reproductive processes). In traditional medicine *C. pallida* is used to treat joint swelling. The *C. pallida* leaves have estrogenic potential as estrogen replacement during menopause. Ethanol extract from *C. pallida* leaves showed mutagenic activity on strain TA98, whereas dichloromethane and stigmasterol fractions were found to be devoid of activity. Stigmasterol is a strong candidate for use in hormone replacement therapy during menopause (Boldrin et al 2013).

5. Inhibits the Tyrosinase Enzyme

Tyrosinase is an enzyme related to the formation of melanin in human skin. The ethanol extract of *C. pallida* seed contains the compound crotalariapallins A-C which shows tyrosinase inhibitory activity (Cheng et al 2021), therefore it has great potential to be developed in the beauty industry as a natural skin whitening agent.

6. Anti Diabetes Mellitus

Diabetes mellitus is the most common endocrine disorder. Ethanol, ethyl acetate, n-butanol and petroleum ether extracts of *C. pallida* leaves have antidiabetic activity in alloxan-induced diabetic rats. The extract produced significant antidiabetic effects on the first, third, fifth and seventh days at 300 mg/Kg body weight. The *C. pallida* extracts, the ethanol extract of the leaves showed highly significant antidiabetic activity comparable to the standard drug (Glibenclamide) (Panda et al 2015b).

7. Anti-cancer

The *C. pallida* is empirically used as a food supplement for cancer treatment by the people of North Sulawesi (Rumondor et al 2017). The results of the Microculture Tetrazolium Technique (MTT) study, acridine staining activity revealed that *C. pallida* coumarin was effective in inducing apoptosis activity in cervical cancer HeLa cells. The activity of coumarin in inhibiting HeLa cell proliferation is influenced by concentration and contact time. Coumarin has apoptotic activity by increasing caspase 3 and 9 and degrading Deoxyribose Nucleic Acid/DNA (avoiding further replication) (Umashankar et al 2015b). Extraction of *C. pallida* leaves and seeds carried out using ethanol, ethyl acetate, n-hexane, and water has antiproliferative activity on MCF-7 breast cancer cells (Rumondor et al 2017).

The bioactivity of *C. pallida* as an anti-cancer is thought to be related to the endophytic fungi it contains. The endophytic fungi in *C. pallida*, namely *Alternaria* sp. has the ability to produce coumarin which has strong in vitro cytotoxic activity so it is used

as a cancer drug (Umashankar et al 2015; Umashankar et al 2015b). Some compounds of *C. pallida* seeds are flavonoids (cropalliflavones A-C), homoisoflavonoids, alkaloids (usaramine-N-oxide and cropallins A-B). The cropalliflavone B compound showed antiproliferative activity against the MCF-7 cell line with an IC50 value of 6.77 µM, and cropalliflavone C showed anti-inflammatory activity, with an IC50 value of 16.07 µM (Hu et al 2017).

C. Toxicity

Some plants used by humans as traditional medicine sometimes have toxic effects, therefore comprehensive information is needed so that negative impacts can be avoided. The *Crotalaria* genus contains toxic compounds, namely pyrrolizidine alkaloids (PA) (Savaris et al 2019). Poisoning caused by consuming *C. pallida* has been reported by Savaris et al (2019), Diaz et al (2003), Gomes et a (2005), Diaz et al (2014), and Prada et al (2020). Most species of the genus Crotalaria cause acute or chronic liver injury (Savaris et al 2019).

Savaris et al (2019) stated that initially *C. pallida* was used as green fertilizer so it was easily spread and could result in contamination of animal feed that was harvested accidentally. The seed of Crotalaria that contaminate soybeans and corn as feed formulations for production animals (Savaris et al 2019). Laying hens fed a diet of *C. pallida* seeds (0, 1, 2, and 3% w/w) for 35 days reduced body weight and consumption feed (Diaz et al 2014). Chicks given feed containing (control, 1.2 and 3% *C. pallida* seeds) for 21 days showed mortality rates of 0, 2.1, 6.2, and 16.7% respectively (Diaz et al 2003). Broiler chickens fed a diet containing *C. pallida* seeds for 28 days showed clinical signs including lack of appetite, ruffled feathers, and brown diarrhea. The following gross lesions were observed: subcutaneous edema, ascites, hydropericardium, yellowish liver with hypertrophy or atrophy and an increased lobular pattern, and an enlarged gallbladder (Savaris et al 2019).

The egg mass production and average egg weight decreased with feeding ≥2% *C. pallida* seeds (Diaz et al 2014). The seeds of *C. pallida* for dehydropyrrolizidine alkaloid content detected usaramine and N-oxide at a total alkaloid concentration of 0.18% (dry weight). Usaramine was also detected in the eggs of all chickens fed *C. pallida* seeds (Diaz et al 2014). Body weight gain and feed efficiency were negatively affected by all *C. pallida* grain inclusion levels, but feed consumption decreased only at 2 and 3% feed levels. Feed levels equal to or greater than 1% *C. pallida* are toxic to growing broiler chickens (Diaz et al 2003).

A trypsin enzyme inhibitor, named CpaTI has been isolated from *C. pallida*. CpaTI weakly inhibits chymotrypsin and elastase and its inhibition of papain, a cysteine proteinase. CpaTI inhibits the digestive enzymes of insects to different degrees (Gomes et a 2005). Pyrrolizidine alkaloids from the Genus Crotalaria have hepatotoxic and genotoxic properties which cause acute poisoning in animals and humans (Prada et al 2020). Several types of pyrrolizidine alkaloids found in the *Crotalaria* genus are the cyclic diester retronecine monocrotaline, crotaleschenine, integerrimine, usaramine, and N-oxide. Seeds and flowers have higher amounts of pyrrolizidine alkaloids than roots and leaves. Due to its 1,2-unsaturated pyrrolizidine alkaloid content, ingestion of *Crotalaria* seeds or other parts via herbal products, infusions, or natural remedies is a serious health threat to humans and livestock (Prada et al 2020).

IV. Conclusions

Crotalaria pallida has bioactivity as: anti-inflammatory, anti-microbial, antioxidant, estrogenic, inhibits tyrosinase, anti-diabetes mellitus, and anti-cancer. The seeds and flowers of *C. pallida* contain dehydropyrrolizidine alkaloids which have hepatotoxic and genotoxic properties which cause acute poisoning of animals and humans.

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