

Morphological, Anatomical Characters and Antimicrobial Activity of *Crotalaria pallida* Aiton.

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Abstract

Crotalaria pallida Aiton. belongs to the family Fabaceae. This plant distributed in Kyaing Tong University Campus Area. In the present study, morphological, anatomical characters and antimicrobial activity were carried out. The morphological characters are herbs to sub-shrubs, leaves trifoliate, leaflet elliptical to obovate. Flowers are yellow and often reddish or purplish brown strike and borne on long racemes, fruits are oblong and 30-40 seeded, seed stalk, reniform, shiny, and olive-green to brown. In anatomical characters, the stomata are anisocytic types, which abundant both surfaces. Uniseriate, unicellular trichomes are present in leaves. The antimicrobial activity against the tests organisms was founded. Especially methanol and water extract was more effective than other solvent but possess the highest against on *Candida albicans* and *Aspergillus flavous*.

Keywords: *Crotalaria pallida*, morphological, anatomical, antimicrobial activity

Introduction

Crotalaria L. is one of the largest genera in the family Fabaceae and comprises some 720 species distributed mainly in tropical and subtropical areas of the southern hemisphere with the principal centre of diversity in the central-south and eastern regions of Tropical Africa (Polhill, 1982, Nafia, 2017). *Crotalaria pallida* Aiton. is popularly known as rattle or rattlesnake due to the sound of their fruits when dry. The plant is grown as a ground cover a green manure crop throughout humid tropics, especially in the inter rows of rubber trees and coconut palms to reduced soil erosion. Flowers are eaten as a vegetable in Cambodia, where the seeds are roasted and grounded for use as a sort of coffee beverage. The roots are sometimes chewed with betel nuts in Vietnam. *Crotalaria pallida* Aiton was reported to be widely used in the southern part of U.S.A as a green manure crop under the name 'giant Striata (Yaradua, 2018).

In traditional medicine, the plant is used to treat urinary problems and fever, a poultice of the roots is applied to swelling of joints and an extract of the leaves is taken to expel intestinal worms (Chong and Corlett, 2009). *Crotalaria pallida* is used in the treatment of diabetic, skin infection, snake bite and stomach ache prevention (Chandan, 2014). It has also been reported to produce the toxic pyrrolizidine alkaloids, monocrotaline and spectabiline, which causes poisoning in livestock and cattle (Williams & Molyneux 1987).

The aims and objectives are the morphological, anatomical characters, to investigation the physicochemical and to determine the antimicrobial activity of *Crotalaria pallida* Aiton leaves.

Materials and methods

The plant was collected from Kyaing Tong University Campus Area during the September-November, 2019. The plant was identified at the Department of Botany, Kyaing Tong University, with the literature of Hooker, 1885, Backer, 1965, Lawrence, 1969 and Hu Qi-ming. (2008). The collected leaves were detached and washed with tap water and dried under shady place good ventilation for 15 days. Then, the dried samples were powdered using kitchen blender and stored in air tight containers for further study.

For microscopic studies, freehand sections of the lamina, midribs, petiole from the fresh specimens were prepared by using the razor blade. Powdered were examined to get standardization for medicine. The free hand sections and powdered samples were

analyzed by using chloral hydrate solution B.P for clearing reagent Metcalfe and Chalk, 1960, Sundara, 2000, Trease and Evans 2002.

Physicochemical investigation of leaves of *C. pallida* Aiton. was made according to The British Pharmacopoeia 1968.

Antimicrobial activities of crude extracts of sample were tested on six pathogenic microorganisms by using paper disc diffusion method described by Cruickshank, R.1975.

Results

Scientific Name	- <i>Crotalaria pallida</i> Aiton.
Vernacular Name	-Taw paik san
Family Name	- Fabaceae
English Name	-Smooth crotalaria, Rattlesnake, Rattlepod

Annual or short-lived perennial herbs to sub-shrubs, an erect, up to 1.5m high. Leaves digitately trifoliate, alternate, leaflet oblong or elliptical to obovate, middle leaflet larger than the lateral leaflet, adaxially glabrous, veins distinct on both surfaces, broadly cuneate at the base and apex obtuse or emarginate and shortly mucronate; margin entire, petiole long. Inflorescences terminal and axillary racemes, 10-40 yellow flowered, bracteole filiform, bract linear, caducous. Calyx campanulate, 5-lobed, deflexed, tubular, pubescent. Corolla yellow, exerted beyond the calyx, standard or vexillum broadly orbicular to oblong, yellow with reddish or purplish striae, glabrous, base with 2 appendages; keel oblong, greenish yellow, with non-twisted prolonged beak, shallowly rounded, marginally pilose at base, standard and keel marked by red-purple striations; wings oblong-lanceolate, yellow, marginally pilose on basal part. Stamen 10, monadelphous, dimorphic, 5 anther long and basifixed, 5 ovoid anther dorsifixed. Ovary oblong, pubescent, unilocular with many ovules on the marginal placentation; style long, incurved; stigma simple. Pods shortly stipitate, oblong-subcylindrical, deflexed, grooved on one side; seeds oblique reniform, 25-40 seeded, shiny, olive green – brown.



Fig 1. Habit, Inflorescences,(standard, wing, keel),Stamens, style and stigma, Fruits
Anatomical Characters of *Crotalaria pallida* Aiton.

Lamina

In surface view, the cuticles were present on both surfaces. The epidermal cells of both surfaces were thin-walled, parenchymatous. Anisocytic types of stomata on the upper and lower surface. Non-glandular (unicellular, uniseriate trichomes) are present on lower surfaces.

In transverse section, the cuticle is present on both surfaces. Cuticle layer was thin. Epidermal cells are parenchymatous with straight anticlinal wall and one-layered thick. Both epidermis are barrel-shaped, thin-walled, compactly arranged. The palisade parenchyma cells found below the epidermis was two-layered thick, vertically elongated, cylindrical closely compacted. The spongy mesophyll cells situate below the palisade.

The vascular bundles of the lateral veins are embedded in mesophyll cells. Xylem composed of tracheids, fibre-tracheids, fibres and xylem parenchyma cells.

Phloem consisted of sieve tubes, companion cells and phloem parenchyma cells (Figures 2, 3, 4).

Midrib

In surface view, the epidermal cells of both surfaces were made up of thin-walled, parenchymatous. They were rectangular and elongated along the length of midrib, non-glandular (unicellular, uniseriate) trichomes were present on lower surface.

In transverse section, the midrib was slightly curved inwards above, with a prominent ridge on the upper surface; both surfaces are covered with thin-cuticle. Epidermal cells are one-layered, rectangular-shaped, compactly arranged, anticlinal walls straight.

The cortex was made up of thin-walled palisade parenchyma cells on upper sides and parenchyma cells on both sides. Parenchyma cells were 7-9 layers in thickness towards the adaxial, 5-9 layers in thickness towards the abaxial side.

The vascular bundles were crescent-shaped and bicollateral type. Xylem cells were radial rows, lignified and thin-walled, composed of tracheids, fibres-tracheids, fibres and xylem parenchyma. The phloem cells were thin-walled and composed sieve tubes and companion cells (Figure.5).

Petiole

In surface view, the epidermal cells of both surfaces were made of parenchymatous cells. They were thin-walled, rectangular to polygonal in shaped. Unicellular, uniseriate trichomes and stomata are present.

In transverse section, the petiole was concave an adaxial side and had cuticle, the epidermal cells were depressed, parenchymatous oval-rounded in shaped compactly arranged, unicellular, uniseriate trichomes were present on both sides of the petiole. Below the epidermis palisade parenchyma cells were one-layers and upper vascular bundles had 8-14 layers of parenchyma and lower vascular bundles had 3-5 layers of parenchyma, the cells were thin-walled, oval-rounded.

The vascular bundles were a medium arc-shaped and bicollateral type usually accompanied by 2 smaller bundles on either side of the grooved adaxial surface and one large bundle. The xylem were lignified thick-walled composed of tracheids, fibres-tracheids, fibres and xylem parenchyma, phloem composed of sieve tubes, companion cells and phloem parenchyma (Figures 6, 7, 8).



Fig 2. Upper epidermal cell of lamina



Fig 3. Lower epidermal cell with trichomes

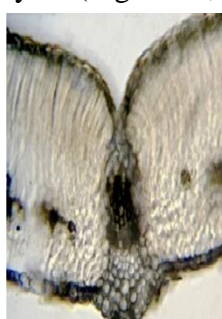


Fig 4. T.S of lateral veins showing vascular bundle



Fig 5. Transverse section of vascular bundle of midrib

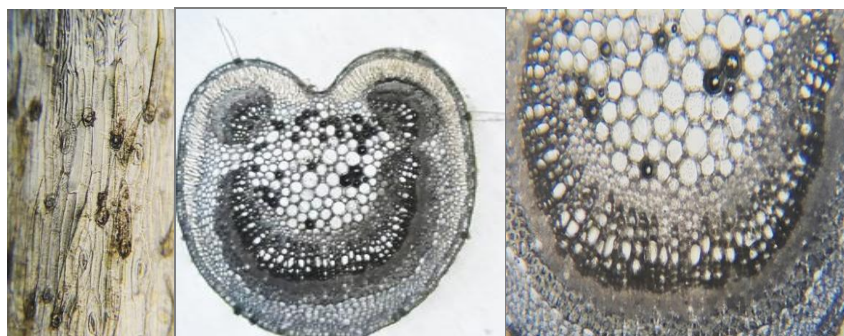


Fig 6. Surface view of petiole with stomata and trichomes

Fig 7. Transverse section of petiole

Fig 8. Closed up view of vascular bundle of petiole

Sensory characters of leaves of *Crotalaria pallida* Aiton.

Color - greenish Taste - a slightly bitter
Odor - pungent Texture-granular and fibrous



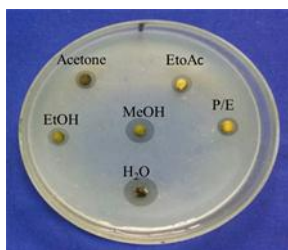
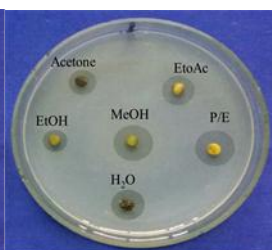
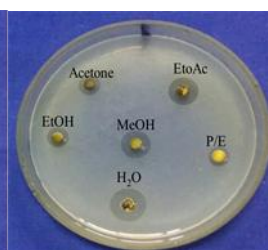
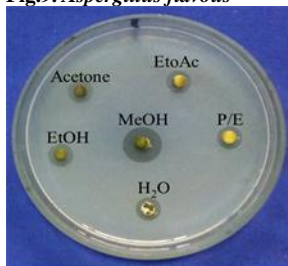
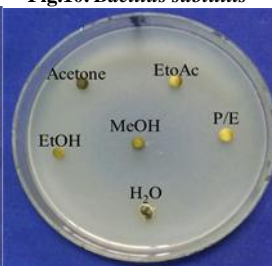
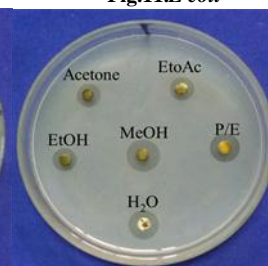
Table 1. Physicochemical Investigation of leaves of *Crotalaria pallida* Aiton.

No	Physicochemical properties	Leaves Average%
1	Petroleum ether soluble content	2.0
2	Ethyl Acetate soluble content	5.0
3	Acetone soluble content	4.0
4	Methanol soluble content	13.0
5	Ethanol soluble content	16.0
6	Distilled water soluble content	18.0

Table 2. Antimicrobial activities of Leaves of *Crotalaria pallida* Aiton.

Solvents	Organisms					
	<i>Bacillus subtilis</i>	<i>Aspergillus flavus</i>	<i>Pseudomonas fluorescens</i>	<i>Xanthomonas Oryzae</i>	<i>Candida albicans</i>	<i>E. coli</i>
Pet-ether	14mm(+)	8mm(+)	-	10mm(+)	8mm(+)	8mm(+)
Acetone	10mm(+)	8mm(+)	-	8mm(+)	10mm(+)	8mm(+)
MeOH	14mm(+)	14mm(+)	-	14mm(+)	16mm(+)	12mm(+)
EtOAc	12mm(+)	8mm(+)	-	8mm(+)	10mm(+)	12mm(+)
EtOH	8mm(+)	8mm(+)	-	8mm(+)	10mm(+)	8mm(+)
D/W	12mm(+)	16mm(+)	-	12mm(+)	8mm(+)	14mm(+)

According to this experiment, the highest antimicrobial activity of methanol and water extract was observed on *Candida albicans*, *Bacillus subtilis*, *Aspergillus flavus*, *Xanthomonas oryzae* and *E.coli*. Various extract did not show antimicrobial activity on *Pseudomonas fluorescens*.

Fig.9. *Aspergillus flavous*Fig.10. *Bacillus subtilis*Fig.11. *E. coli*Fig.12. *Candida albicans*Fig.13. *Pseudomonas fluorescens*Fig.14. *Xanthomonas oryzae*

Discussion and Conclusion

In this study, the morphological characters, anatomical characters, physicochemical investigation and antimicrobial activity of leaves of *Crotalaria pallida* Aiton. were carried out.

Crotalaria pallida Aiton. annual or short-lived perennial herbs to sub-shrubs. Leaves trifoliate. Inflorescences terminal and axillary racemes, 10-40 yellow flowered, standard broadly orbicular to oblong, keel oblong, prolonged beak, standard and keel marked by red-purple striations; wings oblong-lanceolate, yellow. Stamen-10, dimorphic. Pods oblong, sub-cylindrical, grooved on one side; seeds oblique reniform, 25-40 seeded, shiny, olive green-brown. These characters agreed with Dagar 1988, Dassanayake, 1991, Chong 2009 and Michael, 2016.

In anatomical characters, the lamina showed anisocytic stomata on both surface. Unicellular, uniseriate trichomes are present on lower surface. In midrib, vascular bundles are crescent-shaped and bicollateral. In petiole, two smaller bundles were present on either side of the grooved adaxial surface and one large bundle. These characters were agreement with these described by Easu, 1965, Sundra, 2000 and Pandey, 2011.

The physicochemical properties showed that the solubility of the powdered samples were more soluble in D/W, ethanol and methanol than other solvents. Therefore, the leaves of *Crotalaria pallida* Aiton should be extracted with ethanol and methanol for further chemical study and preparation of extracts for pharmacological study.

In this study, the highest antimicrobial activity of methanol and water extract was observed on *Candida albicans*, *Bacillus subtilis*, *Aspergillus flavous*, *Xanthomonas oryzae* and *E. coli*. Various extracts did not show antimicrobial on *Pseudomonas fluorescens*. Nifisa, 2017 reported *C. pallida* is used to antibacterial, anti-inflammatory, antioxidant, estrogenic, mutagenic, thrombolytic activities and it is found to act as HIV-protease inhibitor. Shruthi, 2011 stated that in traditional uses, the leaves of *C. pallida* Aiton. are used as the curative agent of urinary tract infections (UTI) and vermifuge and potential HIV-1 protease inhibitor.

For further research programme, pharmacological activities should be carried out. As *Crotalaria pallida* Aiton possess many medicinal values other bioactive compounds should be isolated from other plant parts.

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