MORPHOLOGICAL, ANATOMICAL CHARACTERS, PHYSICOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITIES OF *CROTALARIA JUNCEA* L.

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Abstract

In the present study, *Crotalaria juncea* L. belongs to the family Fabaceae which is grown in Kyaing Tong University Campus. In the present study, morphological characters, anatomical characters, physicochemical properties and antimicrobial activity were carried out. In morphological characters, erect, simple or branched, appressed hairy, annual herbs, stem conspicuously striated, leaves oblong to oblanceolate. Flowers terminal racemes, bracts and bracteoles linear, corolla golden yellow; pods cylindrical, brown silky. In anatomical characters, the stomata anisocytic types which abundant on both surfaces and unicellular, uniseriate trichomes abundant on lower surface. Leaves and stem shows non-glandular, unicellular and uniseriate trichomes. In physicochemical properties, the aqueous extracts showed more soluble than other solvents. In antimicrobial activities, results revealed that *Crotalaria juncea* L. possess significant antimicrobial activities against *Aspergillus flavous* and *Bacillus subtilis.*

Keywords: Morphological, Anatomical, Physicochemical Properties, Antimicrobial Activities

Introduction

Crotalaria juncea L. is a tropical Asian plant of the legume family Fabaceae. It is considered to have originated in India. It is widely grown throughout the tropics and subtropics as a source of green manure, fodder and liginified fiber obtained from its stem. Sunn hemp is also being looked at as a possible bio-fuel. Doubtfully wild; cultivated extensively for fibre in Parkistan; India; Burma; Malay Isles, Austrial; Russia; introduce in Tropical Africa. Fabaceae consists of about 643 genera, 18000 species. Fabaceae is a very large group with a worldwide distribution (Website-1).

A native of India but widely distributed elsewhere in the tropics. Widely cultivated as a cover crop and for fibre (Dassanayake, 1981). The family Fabaceae as here narrowly defined consists of about 400 genera and 10000 species, widespread in temperate and cold as well as tropical regions. The large genera *Crotalaria*, 500 (Cronquist, 1981).

The plant is native in Asia especially Asia tropical (Bangladesh; Bhutan; India). It is now widely cultivated in the drier areas of the tropics and subtropics and in many temperate areas with a hot summer. It often escaped from cultivation, naturalizes easily and grows in many areas as a ruderal plant. *Crotalaria juncea* L. is recorded in many countries across the African continent from the Atlantic coast to the Red Sea, from Tunisia to South Africa and in the Indian Ocean islands. It is used for medicinal, edible and culinary purposes by many tribal communities. It is traditionally used as blood purifier, abortificient, astringent, demulcent, emetic, purgative, in the treatment of anaemia, impetigo, menorrhagia and psoriasis (Ali Esmail Al-Snafi, 2016).

'Tropic Sun' or Sunn hemp is principally used as a green manure crop for soil improvement. It is an excellent, rapid-growing green manure to be included in rotation with vegetable, ornamental, and other plants to add nitrogen and organic matter to suppress weeds, and to reduce root-knot nematodes. To achieve maximum benefits, plantings should be made at regular intervals in a planned crop rotation scheme (Peter and Robert, 1983).

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Crotalaria juncea L. is grown mainly in India, Brazil, and West Pakistan for its fiber. It is used in the production of twine, rug yarn, tissue paper, fish nets, sacking, canvas, and cordage. *Crotalaria juncea* L. is used as a nitrogen-fixing green manure to improve soil quality, reduce soil erosion, conserve soil moisture, suppress weeds a nematodes, and recycle plant nutrients (Duke, 1983). The aim and objectives are to study the morphological characters of *Crotalaria juncea* L., to know the anatomical characters of *Crotalaria juncea* L., to investigate the physicochemical properties and to examine the antimicrobial activities of *Crotalaria juncea* L.

Materials and Methods

In this study, the specimens of *Crotalaria juncea* L. were collected from Kyaing Tong University Campus. After collected specimens were recorded in detailed for taxonomic description and identification were carried out by (Hooker, 1885, Backer *et al*., 1965, Cronquist, 1981 and Dassanayake, 1991). Microscopical characters of lamina, midrib, petiole and stem were examined by preparing freehand sections from the fresh specimens. The sections were carefully separated with the help of needle, washed with water and cleared in chloral hydrate solution for microscopical studies (Wallis, 1967; Sandara Rajan, 2000 and Trease and Evans, 2002). The diagnostic characters of the powdered drugs were made by using the powders of the leaves.

Physicochemical properties of the leaves of *Crotalaria juncea* L.

The physicochemical properties of the leaves of *Crotalaria juncea* L. were determined according to "The British Pharmacopoeia" 1968 as follows:

Ethanol-soluble matter

Ten gm of air-dried sample was soaked with 200 ml of 95% alcohol in a closed flask for 72 hrs and kept over three nights. The mixture was filtered rapidly taking precautions against loss of alcohol and filtrate was evaporated in a weighed petridish on a boiling water bath, until it was completely dried. The evaporated residue together with the petridish was weighed. The procedure was repeated until a constant weight was obtained. The difference in weights gave the alcohol-soluble extractive value.

Petroleum ether, Ethyl acetate, Acetone, Methanol and Distilled water soluble matter

The above procedure repeated with 200 ml of petroleum ether, ethyl acetate, acetone, methanol and distilled water instead of alcohol.

Antimicrobial Activities of *Crotalaria juncea* L.

Paper disc Diffusion Assay

Isolated bacterial strains grown on nutrient agar were inoculated into 50ml conical flasks containing 10ml of sterile growth medium. Then, they were incubated at 30˚C for 72 hours on a reciprocal shaker at 200 rpm.

Test organisms were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. 3ml of test organisms was added to assay medium, then poured into plates. After solidification, paper discs impregnated with broth samples were applied on the test plates and these plates were incubated for 24-36 hours at 30˚C. After for 24-36 hours, clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive compounds which inhibit the growth of test organisms (Cruickshank, *et al*., 1975).

Assay medium (SY) for test organisms

Agar	- 2.0.g
Sucrose	- 1.0 g
Yeast extract	- 0.3 g
NaCl	- 0.1 g
Distilled waterq	- 100 ml
pH	- 7.0

Results

Scientific name - *Crotalaria juncea* L.

Family name - Fabaceae

Myanmar name - Pike-san

English name - Sunn Hemp, Indian Hemp, Brown Hemp, Madras Hemp,

Diagnostic Characters of *Crotalaria juncea* L.

Annual herbs, to about 2-5 m tall; stems erect, ribbed, subappressed- pubescent; stipules filiform, caducous, leaves simple, oblong to oblanceolate, obtuse at the base, glabrous or sparsely pubescent above, subsericeously beneath; inflorescence terminal, racemose, many-flowered; bracts lanceolate-oblong, acute; bracteoles linear, inserted at the base of calyx; flowers yellow; calyx sub-bilabiate, lobes lanceolate; corolla golden yellow with dark reddish or brown streaks; standard suborbicular, wing oblong, keel falcate, curved, twisted beaked. Stamen 10, monadelphous, anther dimorphic. Ovary oblongoid, unilocular with many ovules on the marginal placentation; style incurved, stigma simple; fruit sessile, oblong-cylindrical, 6-20 seeded; seeds reniform, light brown to black. The results are shown in Fig. 1.

Morphological Characters of *Crotalaria juncea* L.

Habit

Upper surface of Leaves

Lower surface of Leaves

Inflorenscence

Closed up view of flowers

Calyx

Standard

Wing

Keel

Androecium

Gynaecium

Fruits

Seeds

T.S of Ovary

Figure 1 Morphological Characters of *Crotalaria juncea* L.

Anatomical characters of *Crotalaria juncea* L.

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 more lower surfaces than upper surface.

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

epidermises are barrel-shaped, thin-walled, compactly arranged. The mesophyll composed of palisade parenchyma and spongy mesophyll cells. The palisade parenchyma cells found below the epidermis was one-two layered thick, vertically elongated, cylindrical closely compacted. The spongy mesophyll cells situated 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.

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 the midrib, non-glandular (unicellular, uniseriate) trichomes were present on lower surface.

In transverse section, the midrib was curved inwards above, with a prominent ridge on the upper surface, both surfaces are covered with thin-cuticle. Epidermal cells one-layered, rectangular-shaped, compactly arranged, anticlinal walls straight. Collenchyma cells were 1-2 layers situated under the epidermal cells. The cortex was made up of thin-walled parenchyma cells on both sides. The Parenchyma cells were 10-19 layers in thickness towards the adaxial, 5-9 layers in thickness towards the abaxial side.

The vascular bundles are crescent-shaped and collateral 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 of sieve tubes and companion cells.

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 are present.

In transverse section, the petiole is concave an adaxial side and has cuticle, the epidermal cells are depressed, parenchymatous polygonal-shaped compactly arranged, unicellular, uniseriate trichomes are present on both sides of the petiole. Below the epidermis palisade parenchyma cells were one-layer and upper vascular bundles has 15-23 layers of parenchyma and lower vascular bundles has 13-20 layers of parenchyma, the cells are thin-walled, oval-rounded.

The vascular bundles are arc-shaped and bicollateral type. The xylem are lignified thick-walled composed of tracheids, fibres-tracheids, fibres and xylem parenchyma, phloem composed of sieve tubes, companion cells and phloem parenchyma.

Stem

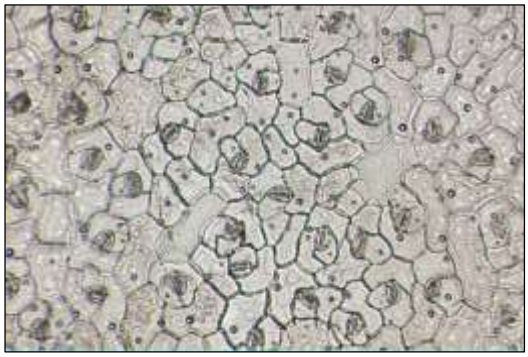
In surface view, the epidermal cells show wavy in outline with a cuticularized outer layer epidermis which is compactly arranged single row of rectangular cells and some cells provide unicellular, uniseriate non-grandular trichomes in young and old stem.

In transverse section, epidermis followed by cortex, which constituted hypodermis with 2-3 layers of collenchymatous cells and also patches of sclerenchymatous cells at the grooved. The cortex is made up of parenchymatous cells. The cells are thin-walled undifferentiated, irregular-shaped in young and old stem.

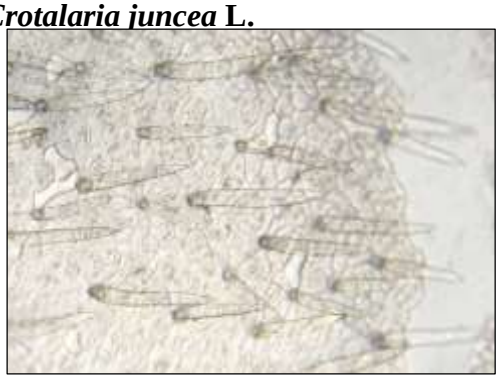
In the vascular bundles, in young and old stem, cortical vascular bundles are present. Endodermis is prominently single-layered composed of elongated irregular cells. Pericycle is single-layered shows presence of stone cells and contains isolated strands of fibers. The cells are

elongated, squarish and oval thick-walled. In xylem, vessels are mostly single or rarely group of 2-3, the smaller one constituting protoxylem lie towards center and bigger one constituting the metaxylem lie away from the center with thick lignified wall in young stem and old stem. Phloem is 3-5 layered, the cells are rectangular, elongated. Fibers thick-walled, radially arranged. Below the xylem 2-3 layers of thin-walled impregnated parenchymatous cells are present, it is compactly arranged, elongated or polygonal. In the centre, pith is large, which composed of compactly arranged parenchymatous cells in the young stem and pith is large, hollow in the old stem. The results are shown in Fig. 2.

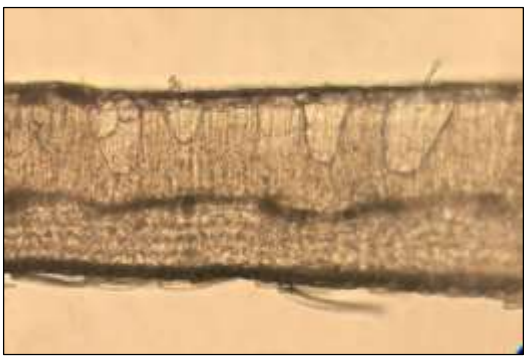
Anatomical Characters of *Crotalaria juncea* L.



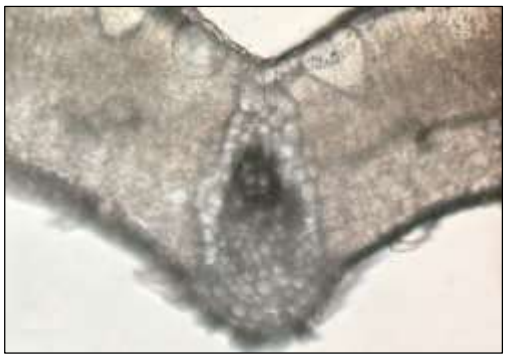
Upper epidermal cells with Anisocytic stomata (X100)



Lower epidermal cells with Anisocytic stomata and trichomes (X100)



T.S of lamina (X100)

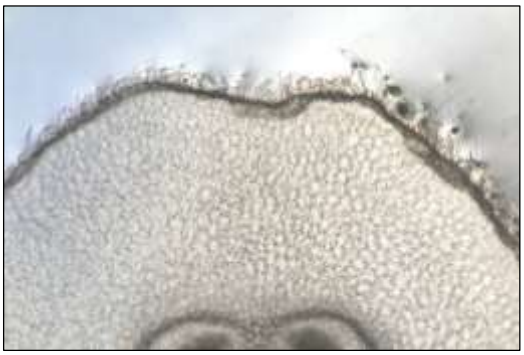


T.S of lateral veins showing vascular bundle (X400)

Surface view of midrib with numerous unicellular, uniseriate trichomes (X100)

T.S of midrib (X40)

Surface view of petiole with unicellular, uniseriate trichomes (X100)

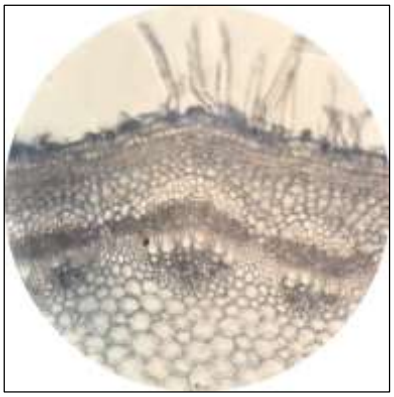


Transverse section of Petiole (X100)

Closed up view of vascular bundle (X100)

T.S of young stem (X100)

T.S of young stem pith (X100)



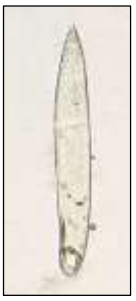
T.S of old stem (X100)

T.S of old stem with hollow pith (X100)

Fibre

Tracheid

Fibre-tracheid



Fragments of unicellular, uniseriate trichomes (X400)

Figure 2 Anatomical characters of *Crotalaria juncea* L.

Table 1 Epidermal characters of *Crotalaria juncea* L.

Scientific name	Stomata		Trichomes	Epidermal cells	
	Upper surface	Lower surface	Non-glandular	Upper	Lower
Crotalaria juncea L.	Numerous (Anisocytic)	Numerous (Anisocytic with trichomes)	Unicellular Uniseriate trichomes	Straight	Slightly wavy

Sensory Characters of the Powdered Leaves of *Crotalaria juncea* L.

Table 2 Sensory Characters of the Powdered Leaves of *Crotalaria juncea* L.

Characters	Leaves
Colour	Greenish
Odour	Aromatic
Taste	Slightly Bitter
Texture	Granular, fibrous

Antimicrobial Activities of *Crotalaria juncea* L. Leaves

In antimicrobial activity, results revealed that *Crotalaria juncea* L. various leaves extracts possess significant antimicrobial activity against *Aspergillus flavous* and *Bacillus subtilis.* The results are shown in Fig. 3 and Table. 3.

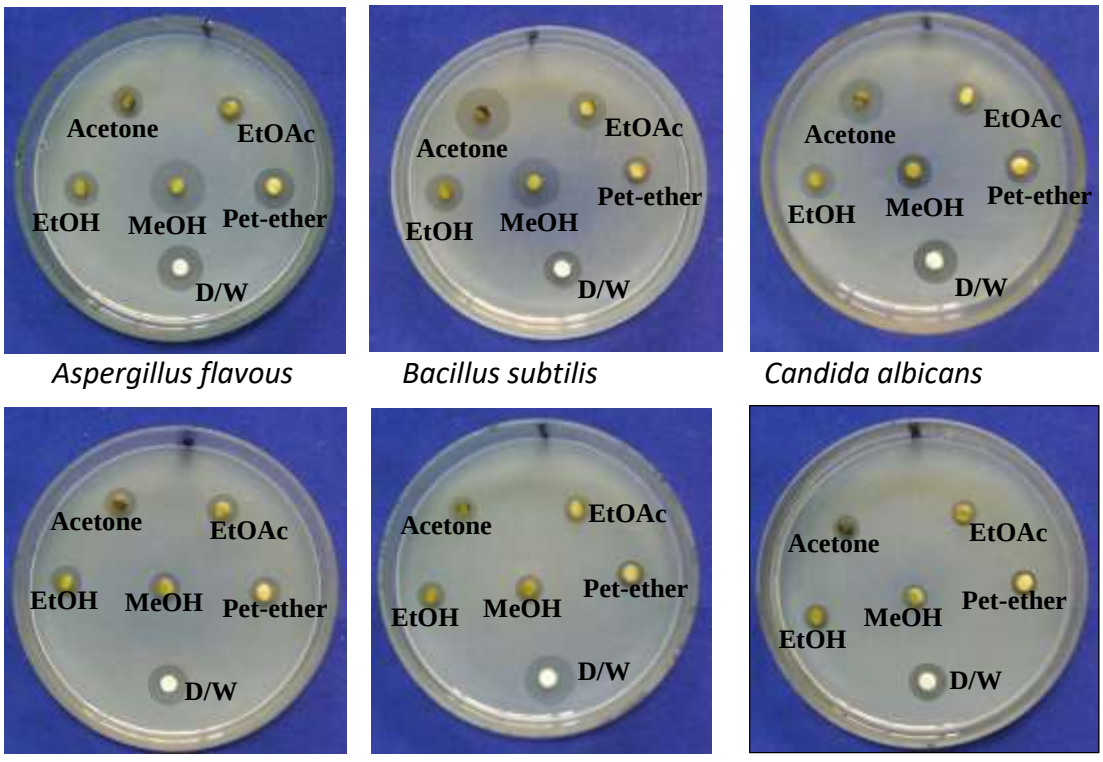


Figure 3 Antimicrobial Activities of *Crotalaria juncea* L. Leaves

Table 3 Antimicrobial Activities of *Crotalaria juncea* L. Leaves

No.	Test Organisms	Pet-ether	Ethyl Acetate	Acetone	Methanol	Ethanol	D/W
1	*Aspergillus flavous*	10	8	**12**	**18**	**14**	**16**
2	*Bacillus subtilis*	**16**	**10**	10	14	8	10
3	*Candida albicans*	12	8	8	8	8	10
4	*Escherichia coli*	8	8	8	8	8	12
5	*Pseudomonas fluorescens*	8	8	8	8	8	12
6	*Xanthomonas oryzae*	8	8	8	8	8	12

Physicochemical Properties of *Crotalaria juncea* L.

Solubility in different solvents

The solubility tests were carried out to find the amount of total solids soluble in solvents. In the *Crotalaria juncea* L. leaves were distilled water more soluble than other different solvents. The results are shown in Table. 4.

Table 4 Solubility Test of *Crotalaria juncea* L. Leaves

Solvents	Pet-ether %	EtOAc %	Acetone %	MeOH %	EtOH %	D/W %
Leaves Average %	3.0	7.0	4.0	14.0	4.0	22.0

Discussion and Conclusion

In morphological study, sunn hemp is erect herbs annual; stems cylindrical and ribbed with short appressed hairs. Leaves alternate, simple; stipules long, slender; petiole long; leaflets oblong-lanceolate, finely appressed pubescent. Inflorescence a leaf-opposed raceme, 6-20 flowered; bracts elliptical. Flowers in terminal racemes, bracts and bracteoles linear, corolla golden yellow, with elliptical standard reddish marked, wings a little shorter than keel, keel long, slightly incurved twisted beak; stamens 10, all joined in a sheath open at base; ovary superior, style curved, stigma small. Fruit a cylindrical pod, short, velvety hairy. Seeds oblique-cordiform, dark brown to black. These characters are in agreement with those given by (Hooker, 1885; Backer *et al*., 1965; Dassanayake, 1981; Ali Esmail Al-Snafi, 2016 and Sonje and Bhuktar, 2016 and Website 2).

In anatomical study, leaves and stems show presence of non-glandular, unicellular, uniseriate trichomes. Trichomes occur on abaxial and adaxial surfaces of leaves, lamina, midrib, petiole and stem (Esau, 1965; Metcalfe and Chalk, 1960; Pandey *et al*., 2011; Sundara Rajan, 2000 and Sonje and Bhuktar, 2016).

Sensory characters showed the colour of powdered leaves were greenish. The odour were aromatic in leaves. The taste of powdered leaves was slightly bitter. The texture was granular and fibrous in leaves.

In this study, the physicochemical properties of *Crotalaria juncea* L. leaves showed pet-ether extract value 3.0, ethyl acetate extract value 7.0, acetone extract value 4.0, methanol extract value 14.0, ethanol extract value 4.0, water soluble extract value 22.0. Sathis *et al*, 2011 and Ali

Esmail Al-Snafi, 2016 stated that ethanol soluble extract value 5.84, water soluble extract value 20.4.

In the antimicrobial activities, petroleum ether and ethyl acetate leaves extracts of *Crotalaria juncea* L. were found more antimicrobial activities in *Bacillus subtilis* than other microorganisms. Acetone, methanol, ethanol and D/W leaves extracts of *Crotalaria juncea* L. against more antimicrobial activities in *Aspergillus flavous* than other microorganisms. *Crotalaria juncea* L. various leaves extracts possess significant antimicrobial activity against *Aspergillus flavous* and *Bacillus subtilis.*

Bhakshul *et al*., 2008 explained that petroleum ether and ethyl acetate extracts of *Candida madurensis var. kurnoolica* leaves were found to be active on the tested microorganisms whereas, the alcoholic extract did not show any inhibitory activity. *Bacillus subtilis*, and *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and *Candida albicans* and *Candida tropicalis* were observed to be sensitive to the tested extracts. These results lend support the usage of *Candida madurensis var. kurnoolica* leaves by the local tribal population in using for wounds and skin diseases against bacterial and fungal infections.

In the further study, this plant review will highlight the chemical constituents will be extracted from methanol extracts and pharmacological activities of *Crotalaria juncea* L.

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