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Phytochemical screening, pharmacognostic study and physicochemical evaluation of leaf of *Crotalaria pallida* Aiton

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ABSTRACT

The present work attempts to carry out preliminary phytochemical screening, detailed pharmacognostic study and physicochemical evaluation of leaf of Crotalaria pallida. Crotalaria pallida Aiton. is a plant with vast medicinal properties. The plant has been traditionally used for the treatment of swelling of joints , fever , vermifuge, skin diseases and also The plant and its parts are reported to posses anti-inflammatory, antimicrobial, antioxidant, antibacterial & antifungal activities. Fresh leaf and dried powder of the leaves were used to studied for macroscopy, microscopy, preliminary phytochemical screening and florescence analysis of powder drug. Other physicochemical parameter were also performed as per WHO guide lines. Leaf constant such as stomatal number, stomatal index, palisade ratio, vein islet number, vein termination number were all so determined. The preliminary phytochemical screening showed presence of alkaloids, flavonoids, terpenoids, saponins, phenols, steroids and tannins in all the solvent extract & carbohydrates abscent in all the extract. These studies provided referential information for correct identification and standardization of this plant material. and detection of adulterants of this plant material.

Keywords: *Crotalaria pallida*, Phytochemical Screening , leaf constant, physicochemical

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INTRODUCTION

The future development of the pharmacognostic analysis of herbal drugs is largely dependent upon reliable methodologies for correct identification, standardization and quality assurance of herbal drugs [1].Evaluation of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulteration. Due to advancement in the chemical knowledge of crude drugs, at present, evaluation also includes method of estimating active constituents present in the crude drug. The detection of active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude plant extracts and is very important with regard to their potential pharmacological effects [2] .The tribal areas of Baipariguda, Koraput (District) of Eastern Orissa. due to its unique varieties geographical and climatic factors has had a rich variety of medicinal plant. *Crotalaria pallida* (family: fabaceae.) also known as jhunjhunuka (Oriya) is frecuntly distributed. And extensively used traditionally by the tribal people. *Crotalaria pallida* Aiton is a species that belongs to the Fabaceae family, popularly known as "rattle or rattlesnake" due to the sound of their fruits when dry [3]. Crotalaria is one of the largest genera in tropical Africa. The genus includes 690 species that are mainly situated in Africa and Madagascar. the. Species have also been found throughout in India. [4] This is an erect shrub, annual short-lived perennial herb of 1.5 m or more tall. Taproot white or brown and stem grooved, solid, glabrous. Leaves trifoliolate, alternate spiral, stalked, leaflets elliptic , more than 2 cm long/ wide, hairy on upper surface, margin entire, apexobtuse base acute, pinnately veined.

Flowers bisexual, grouped together in a terminal raceme, stalked, petals 5, yellow. Fruit a rounded. This species is used 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 fever and its leaves as vermifuge [5]. Mikirs of Assam take about 20 ml. extract of leaves in early morning to kill intestinal worms [6] .Powder of leaf and root bark with the leaf of Wrightia tinctoria & Tragia involucrate is made to a paste with water and applied externally for skin diseases [7] .Pharmacological studies have demonstrated it also presents anti-inflammatory, antimicrobial, antioxidant, antibacterial & antifungal functions [8-11] .*Crotalaria pallida* extracts as a putative HIV-protease inhibitor [12].

MATERIALS AND METHODS

Collection and authentification of plant

The leaves of *Crotalaria pallida* were collected from the tribal belts of the local area of Baipariguda of Koraput district.(India) in the month of November 2011.The plant was identified, confirmed and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-198/11,dated (16.12.2011). Then leaves were shade dried. The dried materials were made into coarse powder and stored in a closed air tight container for further use.

Chemical and Reagents

All the chemicals and reagents used were of analytical grade, purchased from Loba Chemie Pvt. Ltd., Mumbai, India and Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India and All other chemicals reagents used in present work were procured from authorized dealer.

Pharmacognostic study

Leaves were subjected to morphological examinations. Microscopic evaluation of leaf was carried out by taking the transverse sections using standard procedures and then subjecting them to microscopic examination. and were observed under microscope by using (5X,10X,45X) and photographed with (×400) magnifications Various leaf constant like stomatal number, stomatal index, palisade ratio, vein islet number and vein termination number were also determined.by using standard procedure. Different diagnostic features were identified and reported in the results [13,15] .

Extraction of plant materials

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, n-butanol and petroleum ether as solvent. A total amount of 650 g coarse powder was extracted with 1200 ml of each solvent. For each solvent,10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further studies [13,14].

Table no:1 Determination of stomatal number and stomatal index of *C. pallida* leaf

Part used	Stomatal number/mm^2	Epidermal cell/ mm^2	Stomatal index
Lower epidermis	15	106	12.3
	12	108	10.0
	14	110	11.2
	12	106	10.1
	16	105	13.2
Upper epidermis	16	104	13.3
	14	106	11.6
	13	108	10.7
	18	105	14.6
	15	107	12.2

Lower epidermis :
Average stomatal number- ***13.8***
*Average stomatal index-****11.3***

Upper epidermis:
Average stomatal number- ***15.2***
Average stomatal index- ***12.4***

Phytochemical Analysis

The crude ethanol, ethyl acetat, n-butanol and petroleum ether extracts of the leave of *Crotalaria pallida* were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phyto constituents . Chemical tests were carried out For the qualitative determination of phytochemical constituents as per the standard procedure. The various tests and reagents were used result were found in (Table-5) [16,17]

Tableno :2 Determination of Palisade ratio of *C. pallida* leaf

Slno.	Epidermal cells	Palisade cells			Palisade ratio		
		Base	Middle	Apex	Base	Middle	Apex
1	4	25	26	26	6.25	6.5	6.5
2	4	22	24	28	5.5	6.0	7.0
3	4	27	23	26	6.75	5.75	6.5
4	4	24	27	23	6.0	6.75	5.75
5	4	28	22	27	7.0	5.5	6.75

Average palisade ratio: *Base* ***: 6.3 ,*** *Middle* ***: 6.1 ,*** *Apex* ***: 6.5***

Table no: 3 Determination of Vein islet number &Vein termination number of *C. pallida* leaf

Sl no.	No. of Vein islet per mm^2	No. of Vein termination per mm^2
1	9	11
2	10	12
3	11	9
4	8	12
5	11	11

Average Vein islet no. : 9.8 , Average Vein termination no. : 11.2

Table no: 4 Fluorescence analysis of powdered leaf of *C. pallida*

Solvents Used	Observation		
	Day Light	UV 254nm	UV 366nm
Drug powder	Green	Light green	Dark green
Petroleum ether	Pale green	Greenish brown	Black
Ethanol	Pale green	Dark green	Black
Ethyl acetate	Pale green	greenish yellow	Greenish black
Conc. H2SO4	Dark green	Green	Black
50% H2SO4	Black	Black	Black
Conc.HNO3	Greenish brown	Dark brown	Light brown
Conc.HCl	Golden yellow	Greenish brown	Greenish brown
Picric acid	Yelowish green	Green	Black

Table no 5 : Phytochemical screening of *C. pallida* Leaf *extracts*

Phytochemicals	Ethanol extract	Ethyl acetate extract	n-butanol extract	Pet-ether extract
Alkaloids	+++	++	++	++
Flavonoids	+++	++	++	++
Steroids	+++	++	+	++
Terpinoids	+++	++	+	+
Carbohydrates	-	-	-	-
Tannins	+++	++	+	+
Saponins	+++	++	+	+
Phenols	+++	++	+	+

+++, Strong; ++, moderately; +, poor presence; --, absent

Fluorescence analysis

The fluorescence analysis of the powdered drug of leave of *Crotalaria pallida* in various solvents such as etanol, pet.ether, ethyl acetate was performed under normal and Ultra Violet (254nm and 366nm) light and powdered drug reaction with different reagents such as conc. H2SO4, 50% H2SO4, Conc.HNO3 ,Conc.HCl, and Picric acid were were taken and treated individually with desired quantity (1mg) of the plant material result were found in(Table no 4) [18,19]

Physico-chemical evaluation

Analysis of Physicochemical Constants of the ingredient has been done to evaluate the quality and purity of the powder drug. The dried plant material was subjected for determination of physicochemical parameters such as foreign organic matter, all type of Ash Values, alcohol soluble extractive and water soluble extractive, moisture content . Determination of these physicochemical constants was done as per procedures mentioned in accordance with WHO guidelines [18,19,20].

Table no 6 : Physico chemical Analysis of *C. pallida* leaf powder

Analysis parameters	Value (% w/w)
Total ash	10.17 ± 0.21
Acid insoluble ash	2.73± 0.26
Water soluble ash	5.17±0.11
Sulphated ash	3.67±0.14
Alcohol soluble extractives	8.06 ± 0.63
Water soluble extractives	3.51±0.11
Ether soluble extractives	2.6±0.26
Moisture Content	4.32 ±0.12
Foreign organic matter	0.87±0.09

Results expressed as, Mean±SD, n=3

RESULTS AND DISCUSSION

Pharmacognostic study

Macroscopical Evaluation

The leaves of *Crotalaria pallida* Aiton are 3-foliolate; green coloured, petioles *ca*. 4cm long; stipules filiform, caducous. Leaflets are obovate, 3–6x2–3 cm, obtuse - emarginate at apex, entire along margin, cuneate at base, membranous, glabrous above, glaucous and pubescent beneath; petiolule *ca*. 3mm long, woolly. The leaves are slightly bitter in taste, having no odour.

Microscopical Study

The quantitative microscopic study was carried out on the leaves of *Crotalaria pallida* and obtained the following results. The average stomatal number of lower epidermis of the subjected leaf found to be 13.8 and the stomatal index is 11.3, where as in the upper epidermis of the leaf the average stomatal number is 15.2 and the stomatal index is 12.4. The average palisade ratio of the studied leaf found to be at base 6.3, at middle 6.1 and at the apex 6.5. From the study it is observed that the apex of leaf is having higher palisade cells as compared to base and middle part of the leaf. The vein islet number and veinlet termination number of *Crotalaria pallida* found to have values such as 9.8 and 11.2. which were shown in (Table no.1,2,3).

Histological study

Trasver section of leaf of *C. pallida*

PLATE-I

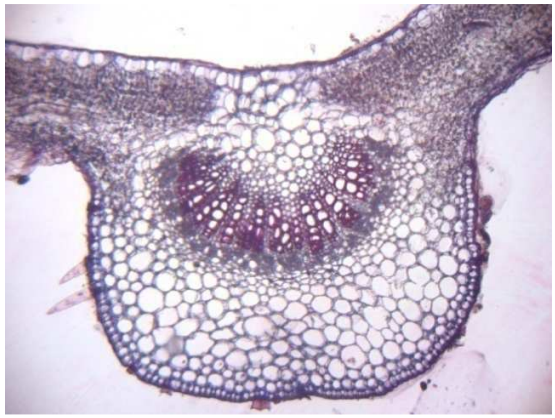

Fig-1(a)

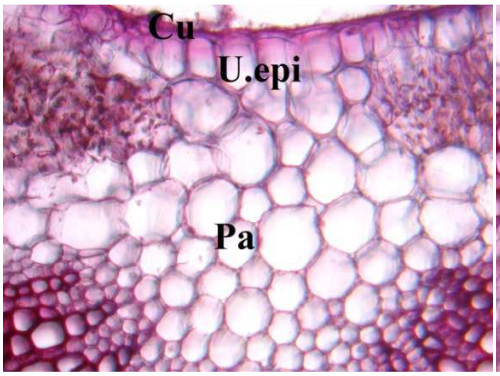

Fig-2(a)

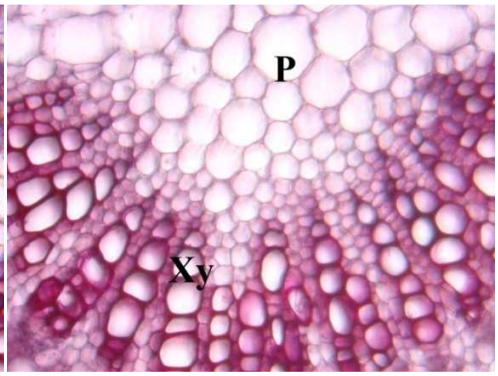

Fig-2(b)

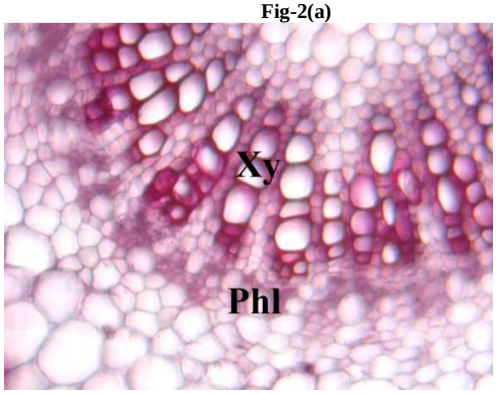

Fig-2(c)

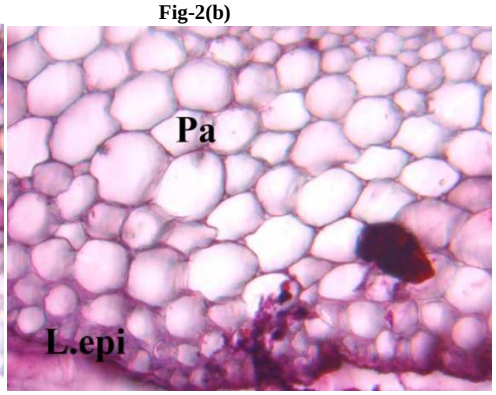

Fig-2(d)

Fig-1(a) –Transverse section of midrib(5X,5X)
Fig-2(a,b,c,d)- Transverse section of midrib (10X,10X)
PLATE-II

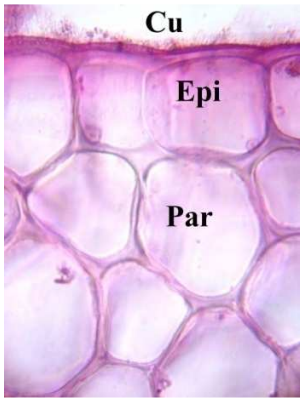

Fig-3(a)

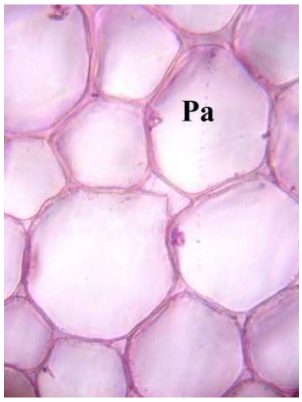

Fig-3(b)

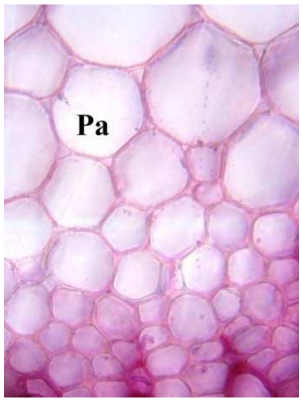

Fig-3(c)

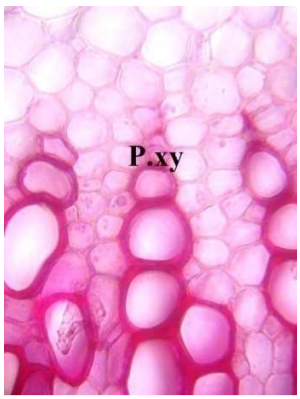

Fig-3(d)

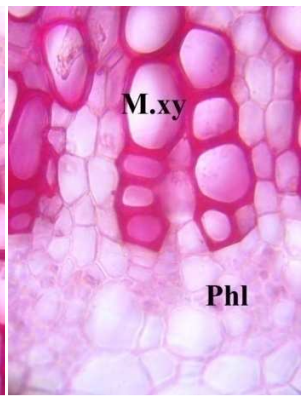

Fig-3(e)

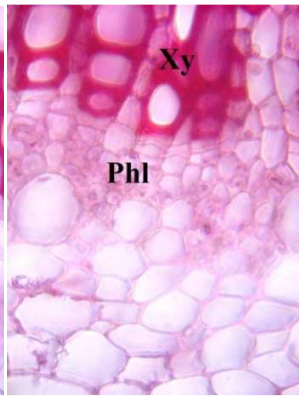

Fig-3(f)

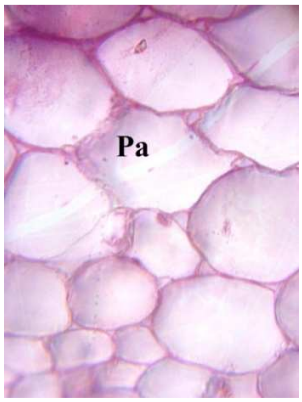

Fig-3(g)

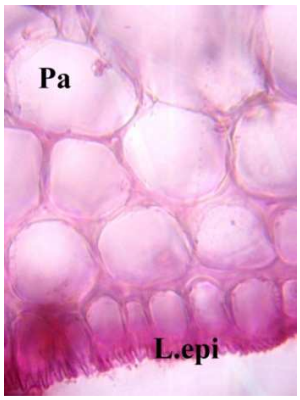

Fig-3(h)

Fig-3(a,b,c,d,e,f,g,h) –Transverse section of midrib(5X,45X)

Trasver section of lamina

PLATE-III

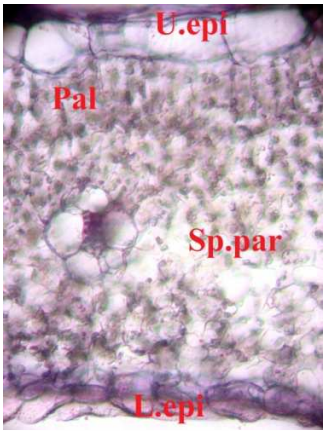

Fig-4(a)

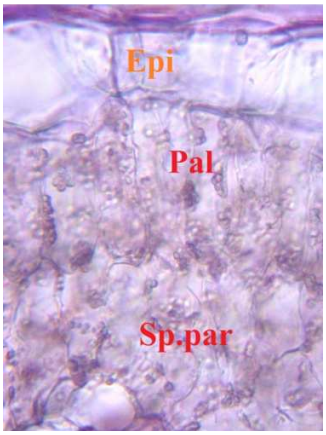

Fig-5(a)

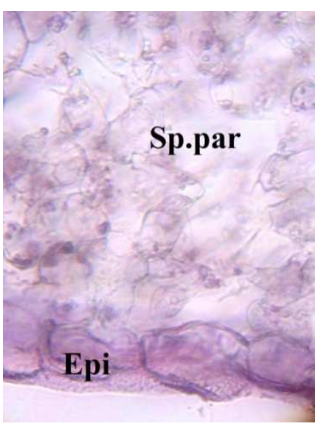

Fig-5(b)

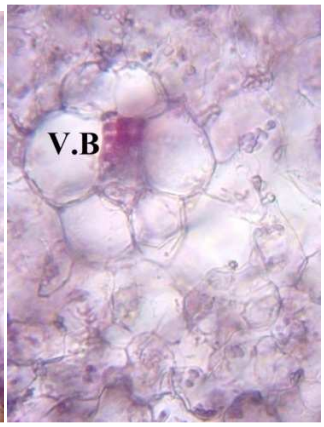

Fig-5(c)

Fig-4(a) –Transverse section of lamina (10X,10X)
Fig-5(a,b,c)- Transverse section of lamina (5X,45X)

Abbreviations *Cu-Cuticle, Epi-Epidermis, U.epi-Upper epidermis, P-Pith, Pa-Parenchyma, Xy-Xylem, Phl-Phloem, L.epi-Lower epidermis, P.xy-Proto xylem.M.xy-Meta xylem, Pal-Palisade cell, Sp.par-Spongy parenchyma, V.B-Vascular bundle.*

T.S of midrib

Transverse section through midrib shows on upper and lower epidermis, vascular bundle and a small central pith between the upper epidermis and vascular bundle. The upper epidermis is formed of single layer of rectangular and squarish parenchymatous cell. Epidermis shows the presence of non lignified covering trichome. The lower epidermis is also formed of single row and consists of comparatively smaller cells than upper epidermis. Upper epidermis is followed by 6-7 layer of parenchymatous cells. Vascular bundle is conjoint collateral. Vascular bundle is arch shaped. Protoxylem lies towards centre and metaxylem towards periphery and the growth of xylem is centrifugal. The central pith traverse through the xylem and form medullary rays. At the centre of the pith large parenchyma with small intracellular spaces are present. Ground tissue is present more towards ventral side and consists of 8-9 layers of parenchymatous cell.

T.S of lamina

The leaf represent a dorsiventral structure. The transverse section passing through the lamina region shows a single layer of rectangular epidermal cells, covered by thick cuticle. Lower epidermis contain cells of small size and covered with cuticle. Mesophyll consists of palisade cells and spongy parenchyma. Palisade cells are elongated and compactly arranged, contain chloroplast. Spongy parenchyma contain loosely arranged parenchymatous cells. Lignified vascular bundle is found in the mesophyll region. Xylem vessels may be spiral or annular.

Phytochemical analysis

The preliminary phytochemical screening showed that the different solvent extracts of *C. pallida*, showed the presence of alkaloids, flavonoids, terpenoids, saponins, phenols, steroids and tannins in all the solvent extract & carbohydrates abscent in all the extract. The ethanol extract yielded strongly, all the phytochemicals followed by petroleum ether,n-butanol and ethyl acetate. The n-butanol extract also yielded all the phytochemicals at poor presence which were shown in (Table no.5).

Fluorescence analysis

The results of the fluorescence analysis of leaves of *Crotalaria pallida* are expressed in (Table no. 4) .The fluorescence analysis of the drug helps to identify the drug with specific fluorescent colours, and also to find out the fluorescent impurities. Thus the study of fluorescence analysis can be used as a diagnostic tool for testing adulteration.

Physicochemical evaluation

The results of the physicochemical parameters of *Crotalaria pallida* leaf powder lie within the limit which is depicted in Table no (1,2,3) this signifies that the quality and purity of raw material was good enough; the results of

foreign organic matter denote presence of any organism, part or product of an organism, other than that named in the specification and description of the herbal material concerned. which was found to be 0.87±0.09 % it indicates that their may be present of part or product of an organism in very less amount. The results of Ash values signify the purity of drug that is the presence or absence of foreign matter such as metallic salt or silica present in the raw material. The total ash usually consists of carbonates, phosphates; silicates and silica which include both physiological ash and non-physiological ash , the values are 10.17 ± 0.21% for total ash. Acid insoluble ash particularly indicates contamination with silicious materials e.g., earth and sand, comparisons of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug which was found to be 2.73± 0.26 %. The water soluble ash was found to be 5.17±0.11 %, this parameter is used to detect the presence of material exhausted by water whereas the value for Sulphated ash was found to be 3.67±0.14 % which is within fairly wide limit. As the ash values of the crude drugs lies with in the fair

limit which signify its quality and purity and gives idea about the total inorganic content. The water soluble extractive value found to be 3.51±0.11 % while the alcohol soluble extractive value was found to be 8.06 ± 0.63 % & ether soluble extractive value was found to be 2.6±0.26 which signifies the nature of the phytoconstituents present in plant. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Deterioration time of the crude drugs depends upon the amount of water present in formulation. If the water content is high, the crude drugs can be easily deteriorated due to fungus and the moisture content of the crude drugs was found to be 4.32 ±0.12 which signify that the powder of the drug was properly dried and properly stored.. The study will provide referential information for the correct identification of the crude drug. which were shown in (Table no.6).

CONCLUSION

Plants serve as vast source for varied phytoconstituents exhibiting varied pharmacological property. Identifying such potential plants is of significance in medicine. So it becomes necessary to study the pharmacognostic characteristic of the plant before its use in the field of research and also in pharmaceutical formulation. Also Pharmacognostic study is the initial step to confirm the identity and to assess the quality and purity of the crude drug. Quality control of crude drugs is very challenging task because of complex nature of chemical constituents.

Moreover it also helps in distinction from other allied species and adulterants. From the present study, it can be concluded that most of the biologically active phytochemicals were present in the different extract of *Crotalaria pallida* leaves. In other words, the results confirmed the presence of therapeutically potent compound in leaf extract of *Crotalaria pallida.*

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