Research Article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Pharmacognostical & Preliminary Phytochemical Investigationof *Cleome Rutidosperma* Aerial Parts

Anuradha.Khuntia*[1] ,SujitKumar Mohanty[1] ,Anindya Bose[2]

[*1]C.E.S.College Of Pharmacy,Chinnatekur,Kurnool, A.P. INDIA.(518003)
[2]SOA University(Department of Pharmacy),Bhubaneswar,odisha

ABSTRACT:-

Cleome rutidosperma ,Linn [Family-Cleomaceae] is claimed to relieve earache,pain.However thorough literature survey indicated that different plants of the same genus available in tropical parts of India have various reported biological activities like antifungal activity,antibacterial activity analgesics, antipyretic,anthelmintic activity ,antimycobacterial activity andcytotoxic activity and CNS stimulant activity etc. The present study was carried out to investigate morphological, microscopical,physicochemical and phytochemical screening of aerial plant. Morphological studies ofleaves, stem, , flower and seed showed the presence of various diagnostic characters. In the microscopicalstudies, leaves showed the presence of vascular bundle, covering trichomes, anomocytic stomata. Ashvalue, extractive value, foreign organic matter and moisture content was determined for quality standard of drugs. The powdered drugswere defatted with petroleum ether and successively extract with different polarity solvent. Phytochemical investigation shows the presence of aminoacid ,terpenes,lipids, steroids, flavonoids . The total ash content of the *Cleome rutidosperma* aerial parts 5.74%.The result of the study could be useful for identification and preparation of monograph of the plant.

KEY WORDS:-aerial parts,*Cleomerutidosperma*,Pharmacognostic; phytochemical

***Corresponding Author-**
Anuradha.Khuntia
Asst.Professor, C.E.S.College Of Pharmacy,
Chinnatekur,Kurnool, A.P. INDIA.518003
E-mail:-anuphchemist@gmail.com
Telephone :-08019913191

INTRODUCTION:-

Cleome rutidosperma grows principally at low altitudes in humid conditions.It occurs up to 400m altitude,in areas with an annual rainfall of 1700-3000nmm occasionally .It is found as a weed up to 1200m altitude.Flowering and fruiting plants can be found throughtout the year,although most abudantly in the rainy season.It is an erect annual herb up to 70cm tall,branched from the base;stem is finely pubescent or glandular pubescent,greenpurplish.Leaves are alternate with 3-foliolate containing petiole up to 7cm long;leaflets are elliptical,glabrous to sparsely setulose-pubescent.Inflorescence is racemose,bracts,similar to leaves.Flowers are small,violet-blue coloured, which turn pink as they age,foundsingly.They are bisexual,regular,4-merous with pedicel up to 3.5 cm long;sepals are linear to lanceolate,2-4.5 mm long,glandular;petals are oblanceolate,6-11 mm long,usuallywhite,sometimespinkish.;stamens are 6 in number; ovary is superior,cylindrical,1-celled.Fruit is cylindrical capsule;with stalk 5-13 mm long,subglabrous,dehiscing with 2 valves;when ripe splts into two scattering many seeds.Seeds are aglobular-reniform,2 mm in diameters,orange-brown-black in colour with fine longitudinal striations and low irregular transverse ridges.

Cleome rutidosperma Linn [Family-Cleomaceae] is claimed to relieve earahe,pain,skindiseases.In Ghana Gabon and DR Congo leaf sap is applied to cure earache and deafness.The plant is used as antimalarial by traditional healers in Cameroon.The plant is used in the treatment of paralysis ,epilepsy,convulsion and spasm.

The literature survey revealed that the systemic evaluation including pharmacognostical study of this plant is still lacking. No scientific parameters are available to identify the true plant material and to ensure its quality. There is a need for documentation of research work carried outon traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. The objective of the present study is to evaluatevarious pharmacognostic standards like microscopy of leaf, stem, ; ash and extractive values,fluorescence analysis , histochemicalcolour reactions of leaf and stemand preliminary phytochemical analysis of *Cleome rutidosperma aerial parts.*

MATERIAL AND METHODS:-

Plant Material;-

The plant was identified by the taxonomists of Botanical survey of India,Shibpur, Howrah and authenticated.A voucher specimen(CR1) has been kept in our research laboratory for future reference. After authentication,fresh aerial parts were collected in bulk from young matured plants,washed, shade dried (11.2% w/w of fresh plant) and then milled into coarse powder by a mechanical grinder. Fresh herb was used to study the macroscopy and microscopy whereas shade dried powder was used for the determination of physicochemical parameter and phytochemical screening.

Macroscopy

The various parts of fresh herb was subjected to macroscopic studies which comprised of organolepticcharacters of the drugs viz., color, odour, appearance, taste, smell, texture, fracture, etc.

Microscopy

Qualitative microscopic evaluation was carried out by taking transverse sections of fresh leaves, and stem of *Cleome rutidosperma.* Free hand sections of the fresh leaves were boiled with chloral hydrate to remove all the coloring matter and then carefully stained with phloroglucinol and HCl (1:1). The sections were transferred to mounted (glycerin) on a slide and a cover slip was placed over it. Powder characteristics of aerial powder were also studied using reported methods.The vital quantitative microscopic leaf constants like vein islet ,vein termination number, palisade ratio,andstomatal index were carried out according to standard method.

Powder study:-

Plants are oven dried at 60°C for 4-6 hrs to make it moisture free and grounded using electric grinder and powder was passed through sieve no. 40. Powder characteristics were studied by standard methods.

Physicochemical parameters

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, were calculated as per Indian Pharmacopoeia.

Preliminary photochemical screening:-

Shaded dried and powdered aerial parts samples were successively extracted with ethanol, petroleum ether, diethyl ether ,ethyl acetate, chloroform andN- butanol. The extracts were filtered and concentrated using vaccum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per Indian pharmacopoeia.

Physicochemical constant and fluorescence analyses:-

These studies were carried out as per the standard procedures(11). In the present study, the aerial part powder was treated with 1N aqueous sodium hydroxide and 1N alcoholic sodium hydroxide, acidslike 1N hydrochloric acid and 50% sulphuricacid. The extracts were alsosubjected tofluorescence analysis in visible/daylight andUV light (254nm & 365nm).

RESULTS AND DISCUSSION:-

Macroscopy:-

Theplant is a procumbent, branching 30-100cm tall perennial herb.Stems are twisted, angular and sparely pubescent.Leaves are 2-3cm long,trifoliate,petiolate,green when young,purplish green when mature.Leaflets are 3 in number,glabrous, chartaceous, rhomboidal-elliptic.Petioluies are 1cm long with both ends acute ,margin is minutely serrulate and revolute,veins are prominently depressed above,elevatedbelow.Terminal leaflets are larger than lateral ,2cm long,and1.1cm wide with 7-10 pairs of lateral veins.Flowers are axillary and solitary .Pedicels are slender ,1.5-1.7 cm long.Sepals are pinkish from outside.Petals are 4 in number,free,linear-lanceolate,membranaceous with 1 mid rib,bluecoloured with caudate apex,entiremargin.Stamens are 6 in number with elongated anthers,saggitate at base.Pistil is glandular hairy.Ovary is subsessile.Style is elongated with disc beaked at apex.Carpels are 2 in number,1 celled.Placentation is parietal.Fruit are linear capsules with apical beaked disk,2.5cm long,1mm thick.Peduncles and stock are 3 cm and 1-4 mm long,respectively .Seeds are 4-25 per capsule,reddish brown to black with white funicular aril,1-1.5mm ,slender,transversely ridged.

Microscopic Character

A) Transverse section of leaf:- Epidermis is single layered ,tightly packed,rectangular cells with upper epidermis consisted of straight or slightly wavy walls and anomocyticstomata.Lowerepidermis consisted

of single layer,tightlypacked,rectangular cells with wavy and with few anomocyticstomata.Numerous unicellular covering trichomes were present on both upper and lower epidermis.The upper and lower epidermis was covered by thin layer of cuticle.

Mesophyll layer lied just under the epidermis layer.It consisted of two layers of elongated upper palisade parenchyma arranged just below epidermis without any intercellular space.Just below the palisade parenchyma 2-3 layers of parenchyma were present.Spongy parenchyma contained isodiametric cells which had intercellular space in between them.

Midrib showed thick walled two layers of collenchymatous cells just above the lower epidermis.The vascular bundle consisted of xylem vessels and phloem fibres.The phloem fibres surrounded the xylem vessels or centroxylic condition.

B)Transverse section of stem:-The transverse section of stem consisted of following three regions;1.Epidermis, 2.Cortex,3.vascular cylinder/stele. They are described below.

1.Epidermis:-It consisted of a single layer of covering cells which were closely packed.The walls were thickened and covered with thin layer of cuticle .Unicellular hair like or trichomes appeared from the epidermis.

2.Cortex:-This region consisted of collenchymas, parenchyma and endodermis.Thecollenchymas cells lied under the epidermis and constituted of three layers of cells.The cell walls were thickened at the cortex.The cells contained chloroplast.Below the collenchymas cells ,there were about four layers of cells called as parenchyma with intercellular spaces.The parenchyma cells made up the major portion of cortex.The endodermis formed the innermost layer of cortex.It was a single layer of tightly packed rectangular cells bordering the stele.

3.Vascular cylinder/stele:-This region comprised of pericycle ,vascular bundle and pith.Pericycle was made up of sclerenchymatous cells which consist of dead fibrecells.The vascular bundle was situated in a ring on the inner side of pericycle.The distinct ring of vascular bundle was seen.Thevascular bundle consisted of three main tissue-xylem,phloem and cambium.The phloem was located towards outside of the vascular bundle and xylem was present towards the centre.The cambium was present in between in xylem and phloem.Interfascicular parenchyma cells were present between each vascular bundle.The pith occupied the central part.It consisted of thin walled hexagonal parenchymatous cells with intercellular spaces.

Table1-Quantitative microscopy of *Cleomerutidosperma*leaf

Leaf constants	Value range	Mean*
Palisade ratio	6-10	8.0
Stomatal index	15-18	16.66
Vein-islet number	6-9	7.2
Vein –islet number	4-6	5.2

Powder Characteristics

Plants are oven dried at 60°C for 4-6 hrs to make it moisture free and grounded using electric grinder and powder was passed through sieve no 40. Powder characteristics were studied by standard methods. The various diagnostic characteristic of powder was fibrous,herbaceous,smooth;colour was light green,taste and odour was characterstic. Microscopic examination of powdershows various characters such as anomocyticstomata,coveringtrichomes,well developed ,thin,long,nonlignified phloem fibres and lignified sclerenchymatousfibres.The walls of epidermal cells were straight or wavy.Some of the epidermal cells showed rounded structure with visible base of the covering trichomes.Fragments of mesophyll showed groups of palisade cells which were elongated with no intercellular spaces.Starch grains and calcium oxalate crystals were absent.

Table.2-Behaviour of the powder of the aerial parts of Cleomerutidospermadifferent chemical reagents.

Treatment	Colour/precipitate	Constituent
Powder as such	Green	-
Powder+Conc. H_2SO_4	Reddish brown	Steroids/Triterpenoids present
Powder+Aq $FeCl_3$	Greenish black	Tannins/Flavonoids present
Powder+ I_2 solution	No blue colour	Starch absent
Powder+Picric acid	No precipitation	Alkaloids absent
Powder+5%Aq .KOH	No change	Anthraquinone glycosides absent
Powder+Aq $AgNO_3$	No precipitation	Proteins absent

Physicochemical Parameters

The physico-chemical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs by African Pharmacopoeia (12).Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign

organic matter such as metallic salts and/or silica (15). The total ash content of the *Cleome rutidosperma aerial parts* is 5.74%.The water soluble ash was 3.10% , acid insoluble ash 2.85%,and sulphated ash 6.81%.The water extractive value is more than that of
ethanol extractive value as shown in table.

Table.3-Ash values of Cleome rutidosperma aerial parts

Type of the ash value	**%w/w**
Total ash	5.74
Acid insoluble ash	2.85
Water insoluble ash	3.10
Sulphated ash	6.81

Table.4 –Extractive values of *Cleomerutidosperma* aerial parts.

Mother extract	**Ethanolic extract fraction**	**%w/w**
Water		19.61
90% ethanol		12.15
	Petroleum ether 40-60^0	3.24
	Diethyl ether	1.09
	Ethyl acetate	0.78
	N-butanol	1.98

Table.5-Histochemical colour reactions of *Cleome rutidosperma*leaf

Reagent	**Colour**	**Inference**
Weak iodine solution	No dark bluish purple colour	Starch absent
A drop of H_2SO_4	Yellowish red colour	Saponins present
Millon's reagent	No red colour	Proteins absent
Phloroglucinol+HCl	Reddish brown colour	Lignin present
Dragendorff's reagent	No orange colour	Alkaloids absent
$SbCl_3$	Reddish pink	Steroids/Triterpenoids present
$FeCl_3$+Na_2CO_3	Bluish colour	Tannins present
5%Aq KOH	Deep yellow colour	Flavonoids present

Preliminary Phytochemical Screening

The powdered plant material was extracted with 90% ethanol using soxhletapparataus.The solvent was removed under reduced pressure,which gave a greenish-black coloured sticky residue (yield- 12% w/w on dried material basis). A portion of dried ethanolic extract wassuspended in water and fractionated successively with petroleum ether (40-600c) ,diethyl ether,ethyl acetate and N-butanol

Table.6-Histochemicalcolour reactions of *Cleome rutidosperma*stem

Reagent	**Colour**	**Inference**
Weak iodine solution	No dark bluish purple colour	Starch absent
A drop of H_2SO_4	Yellowish red colour	Saponins present
Millon's reagent	No red colour	Proteins absent
Phloroglucinol+HCl	Reddish brown colour	Lignin present
Dragendorff's reagent	Very faint orange colour	Very less amount of alkaloids present
$SbCl_3$	Reddish pink	Steroids/Triterpenoids present
$FeCl_3$+Na_2CO_3	No bluish colour	Tannins absent
5%Aq KOH	Nodeep yellow colour	Flavonoids absent

Table .7-Preliminary phytochemical screening of ethanolic extracts and its fractions of *Cleome rutidosperma*aerial parts

Extract/Powder	**Phytoconstituents present**
Water extract	Carbohydrate,Saponins,Flavonoids,Tannins
Ethanolic extract	Carbohydrate,Saponins,Flavonoids,Tannins, lipids,sterols,triterpenoid
Pet-ether fraction of ethanolic extract	lipids,sterols,triterpenoid
Diethyl ether fraction of ethanolic extract	sterols,triterpenoid, Saponins,Flavonoids
Ethyl acetate fraction of ethanolic extract	Saponins,Flavonoids,Tannins
N-butanol fraction of ethanolic extract	Saponins,Flavonoids,Tannins
Powder Drug	Carbohydrate,Saponins,Flavonoids,Tannin,sterol

The yields of the fractions were found to be 26.64%, 8.95%, 6.39% and 16.33% w/w respectively of the ethanolic extract.All the fractions were dried by distillation under reduced pressure.

Preliminary phytochemical screening was performed as per standardized procedure and the various phytoconstituents identified are aminoacid ,terpenes,lipids, steroids, flavonoids.Alkaloids and glycosides were absent in all the extracts.[11,12]

Physicochemical Constant And Fluorescence Analysis:

Thefluorescence method is adequately sensitiveand enables the precise and accuratedetermination of the analyze over asatisfactory concentration range withoutseveral time-consuming dilution steps priorto analysis of pharmaceutical samples .The fluorescence analysis of the powderedcrude drug of *C.rutidosperma* aerial parts ispresented in the table 2 and 3. The aerial parts powder emitted light green under daylight , green under short UV lightand dark green under long UV radiation respectively. Whentreated with aqueous and alcoholic NaOH it remained green in ordinary and short UVlightradiation,bluish green in long UV radiation. Treatmentwith 1N HCL gave blakish brown fluorescence inday light and black in long UV and dark greenish black inshort UV radiation. When treated with 50% nitric acid it shows yellow and greenish yellow in ordinary light and short UV radiation,black in long UV radiation.Alsowhen treated with 50% sulphuric acid it shows redishbrown in ordinary light,,greenish black in short UV radiation and blackish brown in long UV radiation.

The ethanolic extract was brownish green under ordinary day light,green in short UV and fluorescence red rose in long UV.The petroleum ether extract was yellow,pale green, fluorescence deep orange in day light,shortUV,and long UV respectively.The diethyl ether extract was green,palegreen,and fluorescence red in in day light,shortUV,and long UV respectively.The ethyl acetate extract was pale yellow in ordinary light,and short UV;fluorescence red rose in long UV.N-butanol fraction was yellowish red, pale green,andfluorescence white in ordinary day light,shortUV,and long UV respectively.

Table 8-Fluorescence characterstic of the powder of the aerial parts of *Cleomerutidosperma*

Treatment	**Colour in ordinary light**	**Colour under UV light**	
		Short UV(254nm)	Long UV(365nm)
Powder as such	Green	Green	Dark green
Powder+1N NaOH in Methanol	Green	Green	Bluish green
Powder+1N NaOH in water	Greenish yellow	Green	Greenish black
Powder+1N HCl	Blackish brown	Greenish black	Black
Powder+50%HNO_3	Yellow	Greenish yellow	Black
Powder+50%H_2SO_4	Reddish brown	Greenish black	Blackish brown

Table 9-Colour and fluorescence character of ethanolic extracts and its fractions of*Cleome rutidosperma*aerial parts

Parameter	**Ethanolic extract**	**Ethanolic extract fractions**			
		Petroleum ether	Diethyl ether	Ethyl acetate	N-butanol
Colour(day light)	Brownish green	Yellow	Green	Pale yellow	Yellowish red
Short UV(254nm)	Green	Pale green	Pale green	Pale yellow	Pale green
Long UV(365nm)	Red rose(F)	Deep orange(F)	Red(F)	Red rose(F)	White(F)

*F indicates fluorescence.

CONCLUSION

In present investigation various standization parameter such as macroscopy, microscopy, physico- chemical paramenter and phytochemical screening andphysicochemicalconstant and fluorescence analysis was carried which could helpful in authentification of. *C.rutidosperma*.The result of present study will also serve as reference materialin preparation of monograph.

ACKNOWLEDGEMENTS

Author is very thankful to her guide Mr .Anidya Bose and all other staffs of I.P.T.Salipur,Cuttack,Odisha.

REFERENCES:-

1. AsariMM,Ahmedj,Ahmed A, Ansari SH, et al,Pharmacognostic characterization and standardization of Morus alba stem bark,,j Med Aromatic Plant Sci, 2006;28,31-36
2. AbereTA,Onwukaeme DN, EbokaCJ,Pharmacognostic evaluation of the leaves of MitracarpusscaberZucc(Rubiaceae),Tropical Journal of Pharmaceutical Research;6;4 :849-853.
3. BurkillHM,.The useful plants of west tropical Africa..2nded,(Families A-D),Royal Botanic Gardens;UK, 1985:960
4. Bidla. G, et al .Antiplasmodial activity of seven plants used in African folk medicine;Ind J Pharmacol;2004,245-246
5. Dean DA, BurchardKW,Fungal infection in surgical patients.Am J Sur;.1996;171:374-382
6. Indian Pharmacopoeia, Vol II, 4thed, New Delhi; 1996: A- 53-54

7. Iyenger MA, Pharmacognosy of Powdered crude drugs ,2nd ed:Manipal:21-54
8. Kokate C., Purohit A, Gokhale S, PracticalPharmacognosy. 10thed: Vallabhprakashan; New Delhi. 1994:112-120.
9. Khandelwal KR, Pawar AP, Kokate CK et al, Practical Pharmacognosy;NiraliPrakashan: 2001: 19-153
10. Musa KY, Katsayal AU, Ahmed A et al, Pharmacognostic investigation of the leaves of *Gisekiapharmacioides*; *African Journal ofBiotechnology*;5, 2006: 956-957
11. Pimenta AM, Montenegro MC, AraUjo AN et al, Application of sequential injections analysis to pharmaceutical analysis; *Journal of Pharm. Biomed. Annuls*; 2006; 40: 16-34
12. Reddy YSR,VenkateshS,Ravichandran T, et al,Pharmacognostical studies of Wrightiatinctoria bark,pharm.Biol;1999;37:291-295
13. ShahBN,NayakBS,Experimental Pharmacognosy,1sted;M/s S.Vikas& Company:Jalandhar;2008:190-200
14. TreaseGE,Evans WC, Text Book of Pharmacognosy,15thed, Saunders Company Ltd:London:119-159
15. Wallis TE,Textbook of Pharmacognosy ,4th ed,CBSPublishers:New Delhi;572-575