

Department of Botany, University of Jammu, Jammu

Progress Report (upto December 31, 2023)

Title of the project: Cytological, biochemical and molecular characterization of *Cleome viscosa* L., an underutilized species of economic importance.

Sanction No. RA/23/7252-59 dated 23-01-2023

Cleome viscosa L. is an annual rainy season herb belonging to family Cleomaceae. It is commonly known as wild mustard, spider plant or bee plant. Ten populations belonging to four different districts of Jammu and Kashmir were chosen for the present investigation. District wise these segregate into 1:1:2:6 that is, 1 each from Samba and Rajouri; 2 from Kathua and rest from Jammu. These are Mansar Road (Samba), Siot, Sunderbani (Rajouri); Chadwal and Billawar (Kathua); Simbal. Airport, DDOE, Trikuta Nagar, Nagrota and Sidhra (Jammu). Their geo-coordinates are detailed in Table 1.

Table 1. GPS data of *Cleome viscosa* in Jammu and its adjoining districts.

S. No.	Sites	Latitude (N)	Longitude (E)	Altitude (masl)
1.	Samba (SA)	32°56'76"	75°10'21"	384
2.	Airport (AP)	32°40'46"	74°50'34"	288
3.	Trikuta Nagar (TN)	32°41'42"	74°52'46"	308
4.	DDE (DD)	32°43'64"	74°52'27"	316
5.	Simbal (SB)	32°37'58"	74°47'81"	283
6.	Siot, Sunderbani (RJ)	33°06'10"	74°23'48"	523
7.	Sidhra (SD)	32°45'33"	74°55'39"	414
8.	Nagrota (NG)	32°46'53"	74°53'15"	374
9.	Chadwal (CD)	32°47'43"	75°31'33"	324
10.	Billawar (BL)	32°37'48"	75°37'14"	844

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-1-



(a) Simbal

(b) Samba

(c) Nagrota

Fig1 (a-c): Natural habitat of *Cleome viscosa*.



Bbm = 5X Sm



Sbm = 3X Sm



Sm

Fig2: (a-c) Plants of different morphotypes (a) Big branched (Bbm), (b) Small branched (Sbm) and (c) Small (Sm) morphotypes and their respective sizes with respect to Sm.

Morphological studies:

Earlier work had already reported the presence of 4 morphotypes; Big branched (Bbm), Small branched (Sbm), Unbranched (Ubm) and Small (Sm) morphotype in the species. To establish these, transplantation experiments were carried out. The plants were transplanted from their natural

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habitats (Fig1) to the beds in the Botanical Garden, University of Jammu. All these plants adapted well and were analysed for their morphological traits and found to vary in their overall size (large and small) and branching patterns (branched and unbranched) within a population. On the basis of these, three morphotypes namely Big branched (Bbm), Small branched (Sbm) and Small (Sm) morphotypes were found (Fig2). The plants of SD and RJ turned into Sbm and Sm; those of SB turned into Sbm while those of all other accessions turned into Bbm and Sbm. Plants of Bbm of different populations vary in height between 100-150 cm. Those of Sbm are 14-95cm tall and Sm 8-35cm. Details of morphotypes found in different accessions and the range of their heights are summarised in Table 2.

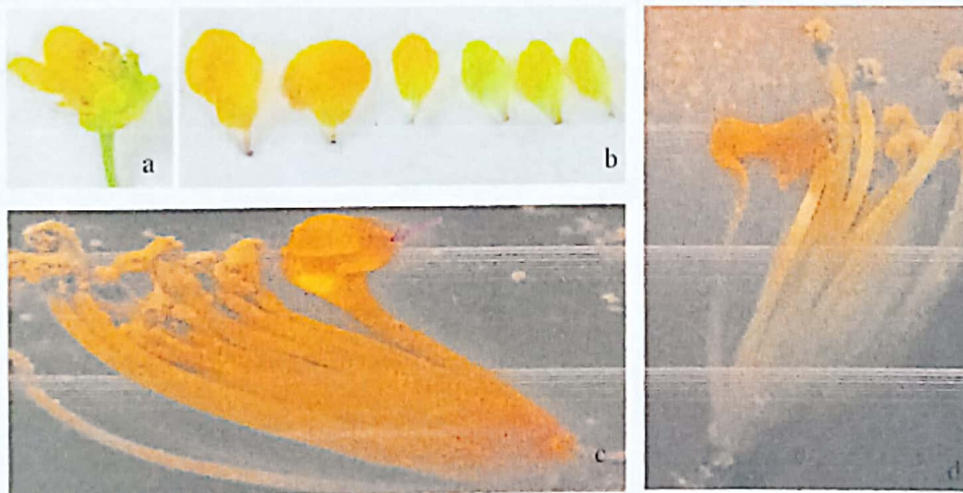
Table 2: Details of morphotypes found in different populations and range of height of their plants.

S.No.	Accession	Height (cm)		
		Bbm	Sbm	Sm
1.	Samba	110-140	60-90	-
2.	Airport	110-150	36-90	-
3.	Trikuta Nagar	100-110	60-90	-
4.	DDE	110	30-95	-
5.	Simbal	-	35-77	-
6.	Dugroin	100-100	40-70	-
7.	Sidhra	-	14-32	12-35
8.	Siot, Sunderbani	-	8-17	8-14
9.	Chadwal	110-146	26-76	-
10.	Billawar	110-145	50-90	-

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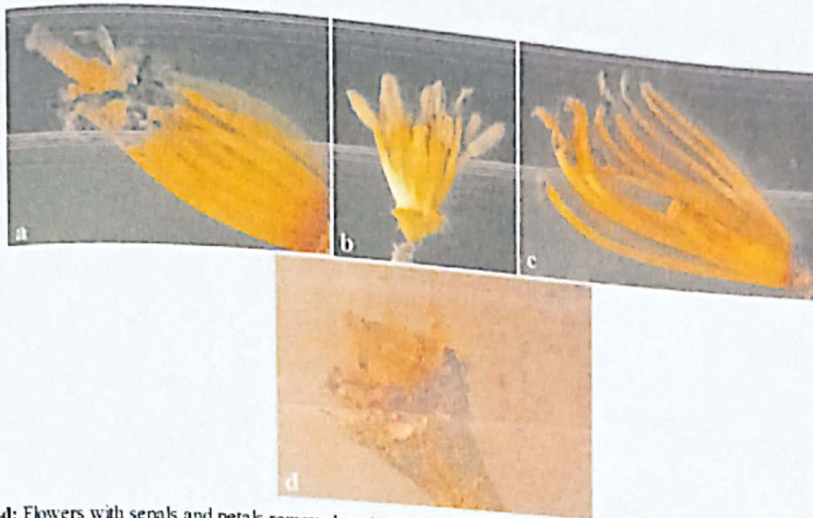
Floral morphology:

Flowers are bright yellow with four sepals and petals, variable number of stamens between 12-20 and a bicarpellary syncarpous, superior ovary. Plants of *Cleome viscosa* are usually andromonoecious i.e., bearing staminate and hermaphrodite flowers. The former lack a functional pistil, while the latter have both the sexual structures and functions. Extensive intra plant floral variation with respect to their size and sex expression has already been reported. For example, hermaphrodite, small hermaphrodite and Staminate flowers with staminodes and pistillode and only Stamminodes with nectary (Figs 4 a-d) have been found. Some other variants were also noticed like flowers having more than four petals (Figs 3 a & b), anther lobes transforming into petals (c & d), etc.



Figs 3(a & b) Flower showing six petals (c & d) stereo-photomicrographs showing petal emerging in place of anther lobe

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-4-



Figs 4 a-d: Flowers with sepals and petals removed to show the sexual apparatus. Accordingly these have been categorized as (a) Hermaphrodite, (b) Staminate hermaphrodite, (c) Staminate with stamens and pedicel, and (d) Staminate with stamens.

Cytological studies:

A total of 785 pmcs were analysed for meiotic details in the male track. All uniformly show normal meiosis irrespective of the morphotype. Pmcs invariably had 10 IIs at diplotene and metaphase I which segregated equally at anaphase I. (Fig 5 a-c)



Fig 5 a-c: Pmcs showing 10 II at metaphase-I (Figs 5a, b of Sm and Sbm plants of Sidhra and Samba respectively) and 10:10 segregation at anaphase I (Fig 5c) of Bbm Kathua.

Biochemical studies:

Phytochemical screening of seeds of *C. viscosa* was carried out in 5 different solvents namely petroleum ether, chloroform, acetone, methanol and distilled water in Bbm and Sbm. The seed extract showed the presence of alkaloids, glycosides and sterol. Presence of these three

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phytochemicals was observed in chloroform, acetone and distilled water, glycosides and sterols in petroleum ether and sterols in methanolic extracts. (Table 3, Fig 6)

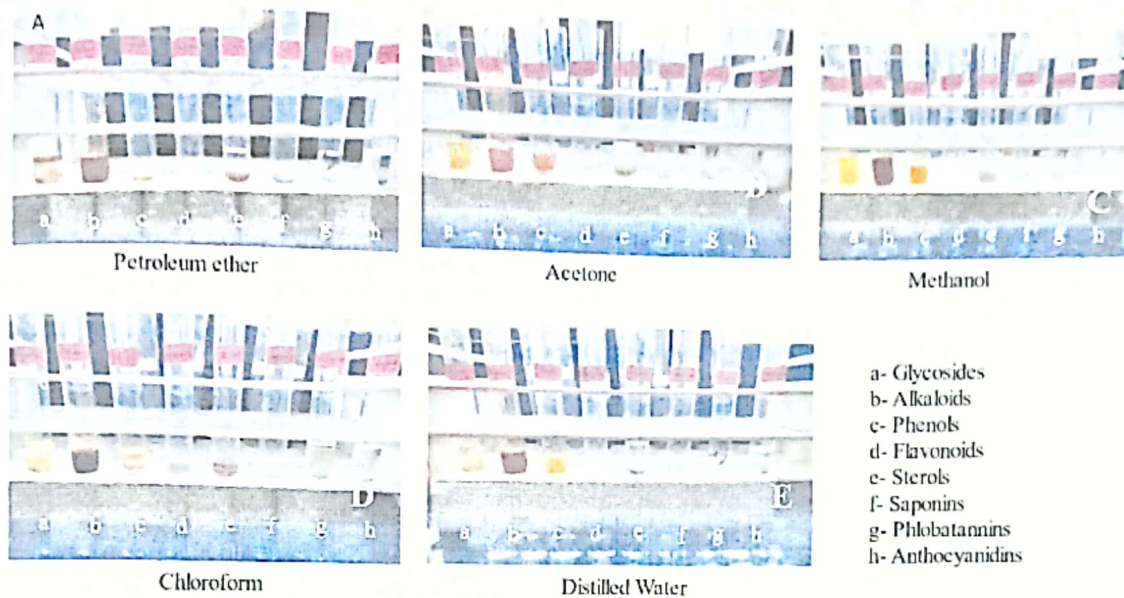


Fig 6 A-E: Results of biochemical analysis on seed extracts in different solvents *C. viscosa*

Table 3. Phytochemicals in seeds of *C. viscosa* and their varying intensities

S.No.	Phytochemical	Intensity of compounds in different solvents									
		Petroleum Ether		Chloroform		Acetone		Methanol		Distilled water	
		Bbm	Sbm	Bbm	Sbm	Bbm	Sbm	Bbm	Sbm	Bbm	Sbm
1.	Glycosides	++	++	++	++	+	+	-	-	+	+
2.	Alkaloids	-	-	++	++	+	+	-	-	+	+
3.	Phenols	-	-	-	-	-	-	-	-	-	-
4.	Flavonoids	-	-	-	-	-	-	-	-	-	-
5.	Sterols	+	+	+	+	+	+	+	+	+	+
6.	Saponins	-	-	-	-	-	-	-	-	-	-
7.	Phlobatannins	-	-	-	-	-	-	-	-	-	-
8.	Anthocyanidins	-	-	-	-	-	-	-	-	-	-

HPLC:

Plants of different morphotypes were chosen and its roots, leaves and seeds were collected separately, washed and shade dried for 10-12 days under room temperature. Two gram of dried root, leaf and seed tissue was homogenized using mortar and pestle into fine powder (Fig 7a,b). This fine powder of different material was immersed into 25 ml of methanol and kept for 24 h (Fig7d) with constant shaking. Following incubation the extracts were filtered using Whatman's filter paper no. 42. The filtrate so obtained was dried to a semi-solid form (Fig 7c). The working solutions for HPLC were obtained by dissolving 6mg of extract in 1ml of methanol and then filtered through Millipore membrane filter assembly. A total of 18 samples (3 samples of root, leaf and seed from each morphotype in methanol) each in 10 μ l injection volume were subjected to HPLC analysis. Nevirapine was used as standard.

GC-MS:

The same working solutions prepared for HPLC were used for Gas chromatography-mass spectrometry (GC-MS). The injection volume used was 1 μ l for GC-MS.

All these samples prepared were sent to Central University of Punjab, Bathinda to carry out HPLC and GC-MS using their apparatus and the results are awaited.

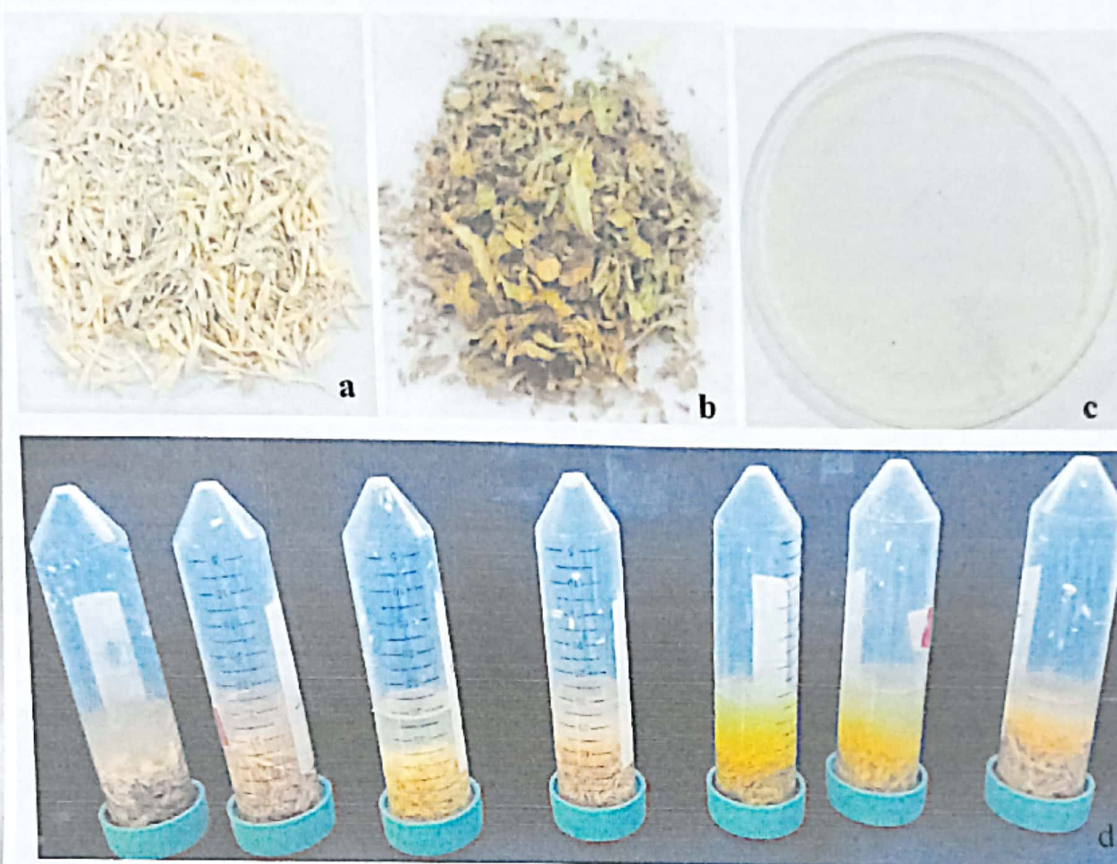


Fig 7 (a, b) Dried powder of roots and leaves; (c) filterate dried to semi-solid form on petriplate; (d) Extracts kept for incubation for 24 h.

Future Plan of work:

Variability in the nevirapine level in different morphotypes will be assessed using HPLC

Genetic variability will be analysed using ISSR markers.

Data so generated will be compiled and analyzed to narrow down to elite genotype (s) for further improvement and selection.

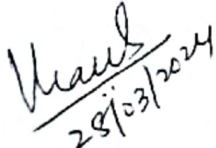
Contribution of seed grant in facilitating this study has been considerable. HPLC and GC-MS for biochemical profiling could be carried out and all the chemicals required for assessing molecular variability of the germplasm could be purchased.

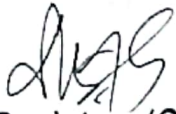
Utilization Certificate

1. Title of the project : Cytological, biochemical and molecular characterization of *Cleome viscosa* L., an underutilized species of economic importance.
2. Sanction Order no. : RA/23/7252-59 dated 23/01/2023.
3. Name of the PI : Prof. Veenu Kaul
4. Department : Department of Botany, University of Jammu
5. Total Project Cost : Rs. 2,00,000
6. Statement of Expenditure:

Head	Total grant released	Total expenditure incurred (INR)	Unspent Balance (INR)
Hiring of Services	75,000.00	74,634.00	366.00
Consumables/ Chemicals/Glassware	80,000.00	80,000.00	0.00
Contingency	30,000.00	29,996.00	4.00
Field work	15,000.00	15,000.00	0.00
Total	2,00,000.00	1,99,630.00	370.00

Certified that out of Rs. 2,00,000 (Two lakhs only) of Grant-in-aid, sanctioned vide order no. reappropriated order no: RA/23/7252-59 dated 23/01/2023 during the year 2023 in favour of **Prof. Veenu Kaul (PI)**, a sum of Rs. 1,99,630.00 has been utilized for the purpose of research for which it was sanctioned and that balance of Rs. 370.00 remained unutilised.


28/03/2024
Signature
PI
Professor of Botany
Department of Botany
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Deputy Registrar (Grants)
Signature
University of Jammu
Deputy Registrar
28/3/24


Joint Registrar (Finance)
University of Jammu
Signature
Joint Registrar
28/3/24