Simultaneous Quantification of Syringic Acid and Kaempferol in Methanolic Extract of *Cleome viscosa* Linn. by Using Validated HPTLC-Densitometric Method

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ABSTRACT

Cleome viscosa Linn. (Capparaceae) commonly known as "Hurhur", is a traditional medicinal plant used in the treatment of various ailments such as fever, inflammation, liver disorder, ulcer stomachache etc. In the present study, pharmacognostical and pharmacological evaluation of *Cleome viscosa* were done, on the basis of traditional claim. A validated HPTLC method was also developed for the simultaneous quantification of bioactive compounds i.e. syringic acid and kaempferol in *C. viscosa*. The chromatogram of syringic acid and kaempferol were developed by using tertiary mobile phase toluene: ethyl acetate: formic acid (7: 3: 0.5) and scanned at 278 nm and 370 nm. The developed method was found to be specific, linear, precise and accurate as per the standard protocol of ICH guidelines. *In-vitro* antioxidant, anti-inflammatory and anti-diabetic potential of the species were evaluated and found that *C. viscosa* have satisfactory potential, may be due to identified marker compounds. This study will be helpful in future for standardization, quality control and confirmation of the right plant material and monitoring of batch to batch consistency of finished herbal products using *C. viscosa* as an ingredient.

Key words: *Cleome viscosa*, HPTLC, Syringic acid, Kaempferol, *in-vitro*.

INTRODUCTION

Cleome viscosa Linn. (Capparidaceae), commonly known as "Hurhur/Jangliharrar" in hindi, "Varada/Pasugandha" in Sanskrit and "Wild mustard" in English. It is distributed in Sri Lanka, Bangladesh, Pakistan, Thailand, China, Africa and India. In India, the species is found in Uttar Pradesh, Madhya Pradesh, Bihar, Chhattisgarh, Bengal and Odisha.[1] *C. viscosa* is commonly grow in the outer layer of agriculture field and also found on road side. Traditionally, *C. viscosa* is used in many ailments such as fever, inflammation, liver disorder and diarrhea.[2] Tribal communities use root extracts of *C. viscosa* for the treatment of fever and leaf extracts for wounds, ulcer and ear pain.[3] Young leaves were also used in culinary purposes having sharp mustard flavor.[4] *C. viscosa* is also used in stomachache, as laxative, diuretic, in itching and leprosy.[5] The species also exhibit hepatoprotective activity,[6] antimicrobial activity,[7] antifibrotic effect,[8] antioxidant activity,[9] antipyretic activity, anthelmintic activity, anti-diarrheal activity, analgesic activity and blood related disorders.[10] The seed of *C. viscosa* has shows the immuno-modulatory activity.[11]

Syringic acid (O-methylated triydroxybenzoic acid) a naturally occurring derivative of gallic acid and kaempferol (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one), is a therapeutically vital compound that has already been reported in a number of plants. Syringic acid is well known for its pharmacological activity as an antioxidant to scavenge free radicals. Kaempferol is also reported to

have antioxidant, anti-inflammatory, neuroprotective, antianxiety and cognitive-enhancing effects. *C. viscosa* contains various class of chemical constituents like fatty acids, volatile oils,[12] macrocyclic diterpene,[13] triterpenoids,[14] coumarinolignoids glycoflavanones,[15] naringenin glycoside,[16] kaempferol, quercetin, cleomiscosin A, B, C, D, cleosandrin, cleomolide,[17] which play a key role in the biological potential of the species.

The aim of current study was to develop HPTLC method for the simultaneous quantification of bioactive compounds i.e. syringic acid and kaempferol and evaluation of traditional claim by *in-vitro* analysis.

MATERIALS AND METHODS

Plant Collection and sample preparation

Cleome viscosa (root) collected from Lucknow, UP in December, 2018 and passport data sheet prepared. Collected plant sample was authenticated by Senior Principal Scientist, CSIR-National Botanical Research Institute, Lucknow, UP. After proper authentication, the plant specimen was deposited in the herbarium department of the Institute (Figure 1). (Herbarium No-305997).

The coarsely powdered root parts of plant sample (2 gm) were extracted (cold maceration) with methanol for 24 hr at room temperature (25 ± 2°C). Extraction was repeated thrice, filtered and pooled filtrate was dried in rotatory evaporator (Buchi, USA) under standard conditions of temperature (55 ± 2°C) and pressure (40 mbar) and lyophilized (Labconco, USA). The extractive yield was calculated (%) on dry weight basis.

Chemicals

Kaempferol (97%), syringic acid (95%), 1-1-diphenyl-2-pic-rylhydrazyl (DPPH), α-amylase, 3, 5-Dinitrosalicylic acid (98%), starch soluble, iodine (99.99%), aluminium chloride (99.99%), sodium carbonate (99%), Folin's reagent (97%), toluene, ethyl acetate, formic acid were purchased from (Sigma-Aldrich, India). TLC silica gel 60 F_{254} plates were purchases from Merck, Darmstadt, Germany. All other solvents and chemicals (AR grade) are obtained from SD Fine Chemicals, Mumbai, India.

Physicochemical screening

Physicochemical parameters i.e. loss on drying (LOD), total ash, water soluble ash, acid insoluble ash and extractive values (hexane, alcohol and water, hydro-alcohol soluble extractives), were evaluated and quantified.[18]

Phytochemical analysis

Phytochemicals analysis such as total phenolic, total flavonoid,[19] total tannins,[20] total sugar and total starch content were quantified by spectro-photometric method.[21]

Anatomical description

Freshly collected roots are preserved in 70% ethyl alcohol for anatomical studies. Section was cut by blade and double stained with safranin and fast green in 50% and 90% ethanol respectively. After that section was fixed with Canada balsom fixative on glass slide, observation and photomicrograph was taken by digital microscope, Nikon, Japan (Model-Eclipse Ci).[22]

Powdered root (40 mesh) was taken on microscopic slide, 1-2 drop of safranin (1% solution of safranin in 50% alcohol w/v) with glycerine were added and mounted with a coverslip and then analysed under light microscopy. Photomicrographs were taken with Nikon, Model Eclipse-Ci upright microscope with DS-Fi2 camera.[18]

Quantification of chemical compounds through HPTLC

Sample and standard solution

The stock solution of sample (*Cleome viscosa*) and standards (syringic acid and kaempferol) 10 mg/ml and 1 mg/ml respectively were freshly prepared in analytical grade methanol. A stock solution of syringic acid and kaempferol were diluted with same solvent to obtain a six working solutions of concentration ranging from 200, 400, 600, 800, 1000 and 1200 μg/ml for further analysis. Before application, the solutions were filtered through a 0.45 μm Millipore membrane filter (Pall, USA).

Apparatus

A CAMAG automated thin layer chromatography (TLC) sampler was used to dispense the aliquots of the standard solution and sample by using Vision CATS software. The plates were developed in CAMAG automatic developing chamber (ADC-2) (20 cm × 20 cm) in standard developed condition. The slit dimensions were 5.0 mm × 0.30 mm and scanning speed was 100 mm/s. Scanning of bands was performed using Camag TLC Scanner 4 in ultraviolet absorbance mode by using deuterium lamp for UV mode.

Chromatographic conditions

Chemical profiling and method optimization for simultaneous quantification of syringic acid and kaempferol were carried out on 20 cm × 10 cm TLC precoated glass plates with 0.2 nm layer thickness of higlachrosep nano silica/UV_{254}. Tracks (standard and sample) were applied as 8 mm bandwidth using CAMAG Automatic TLC Sampler 4 (ATS-4) (CAMAG,

Switzerland) 10 mm from X-axis and 18 mm from Y-axis, under a flow of N_2 gas. The development of HPTLC plate was done by using mobile phase toluene: ethyl acetate: formic acid (7:3:0.5 v/v/v) in automatic developing chamber 2 (ADC-2) (20 cm × 20 cm). The saturation time of chamber was conditioned and optimized to 30 min at room temperature (25 ± 2°C) and relative humidity (55 ± 2%) for better resolution with mobile phase vapors. TLC plate approximately 85 mm height from the point of application (total length run by mobile phase) was allowed to develop. After development, the plates were air dried for 10 min and scanning was performed using CAMAG TLC Scanner at λ_{max} of 278 nm for syringic acid and kaempferol in UV absorbance mode and reflectance mode operated by Vision CATS software. Quantification was performed using calibration curve between peak area Vs concentration of standard marker using regression analysis in the range of 200–1200 ng/band. Images of TLC plate were taken at two wavelengths, *i.e.*, 254 nm and 366 nm.[23]

Method validation of method

In the developed experimental conditions, the HPTLC method for simultaneous quantification of syringic acid and kaempferol includes evaluation of the following performance parameters such as specificity, linearity, sensitivity, accuracy, recovery, precision and robustness as per the standard protocol of ICH guidelines.

In-vitro biological evaluations

In-vitro antioxidant activity

Total flavonoid and phenolic content was estimated[18] and expressed in terms of mg/g of QE (Quercetin Equivalent) and mg/g GAE (Gallic Acid Equivalent) based on calibration curve of quercetin and gallic acid as standard. The antioxidant potential was analyzed via DPPH radical scavenging assay[24] and 2 deoxy ribose.[25]

In-vitro anti-inflammatory assay

The inhibition assay was performed using BSA method.[26] % Inhibition concentration was calculated by using the formula.

$$\text{Inhibition activity (\%)} = \frac{\text{Abs (Control)} - \text{Abs (extract)}}{\text{Abs (Control)}} \times 100$$

In-vitro antidiabetic activity

In-vitro antidiabetic potential of the species was evaluated by using alpha amylase inhibition assay (3, 5-Dinitrosalicylic acid method)[27] and Starch-iodine

method.[28] % Inhibition concentration was calculated by using the formula.

$$\text{Inhibition activity (\%)} = \frac{\text{Abs (Control)} - \text{Abs (extract)}}{\text{Abs (Control)}}$$

RESULTS AND DISCUSSION

Macroscopic and microscopical characters

The fresh leaf of *C. viscosa* contains green coloured 3-5 leaflets, 2-7 cm in length and 1-4 cm in width which is palmately compound and alternate; leaf stalk is 10-40 mm in length, carries hairs, acute apex with obovate to lanceolate margin with round base. Yellow coloured, bisexual flowers, fruits are cylindrical in shape, capsules are erect with many seeds which is seeds reddish brown coloured, sub-orbicular with narrow cleft covered with strong cross ribs and faint concentric ribs, sparsely to un-branched, angular stem with hairs; Long tap roots contains some secondary roots. A plant consist distinctive smell with bitter taste and is sticky in nature (Figure 1).

T.S. of root is circular in outline; the outer most layers is thick walled with cork cells and cortex consists of thin walled, mostly oval shaped parenchymatous cells; Major portion of the section is occupied by well developed vascular tissues. Secondary xylem parenchyma consist meta-xylem and proto xylem; uni to biseriate medullary rays are also present (Figure 2).
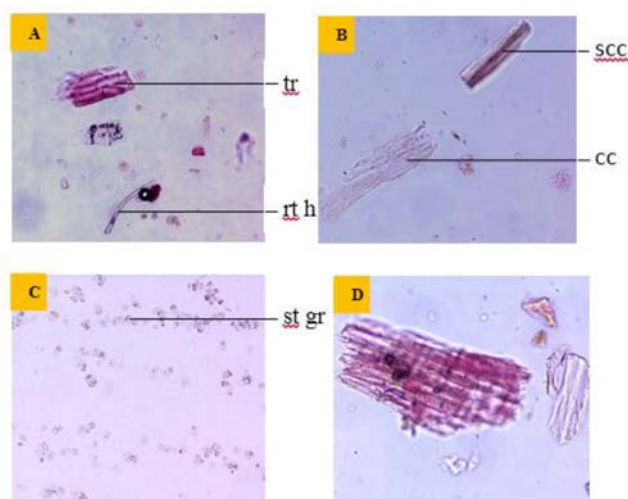

Figure 1: Flowering twig of collected *Cleome viscosa* (root).
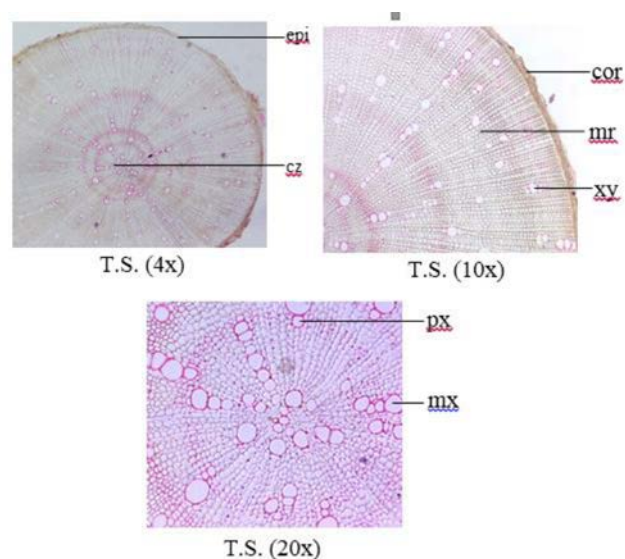
Figure 2: Anatomical description of *Cleome viscosa* root.

Abbreviation: epi- epidermis; cz-central zone; cor-cortex; mr- medullary rays; xy- xylem; mx- meta xylem; px- proto xylem.
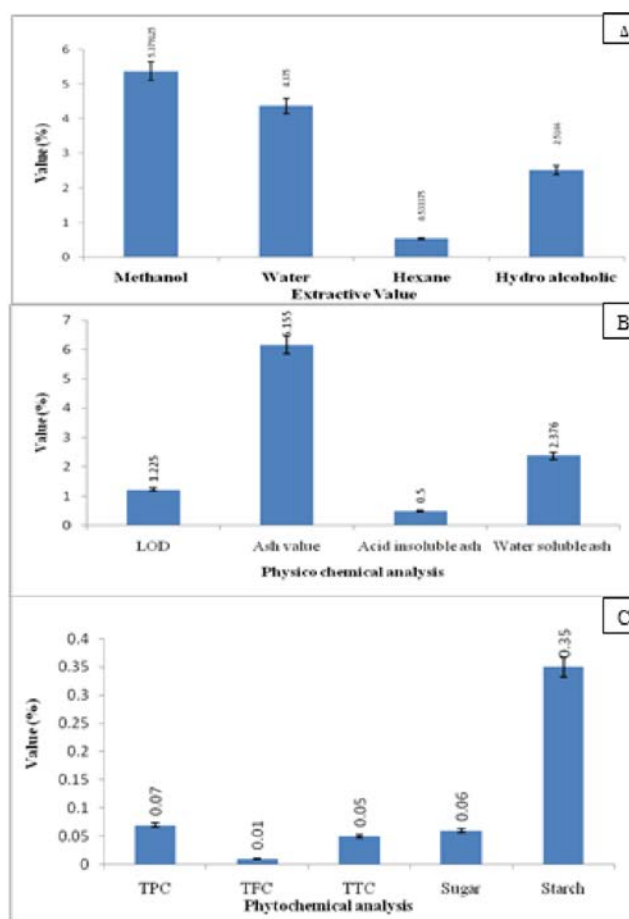

Figure 3: Powder characteristics of *Cleome viscosa* root. (A) tr-Tracheids, Rt h- Root hairs; (B) cc-Cork cell with phelloderm, scc- sclerenchymatous cells; (C) st gr- Starch grains; (D) Medullary rays crossing the parenchyma

Figure 4: Analysis of extractive value (A), physico-chemical parameter (B) and phytochemical analysis (C) of *Cleome viscosa* (root).

Abbreviation: LOD-Loss on drying, TPC-Total phenolic content, TFC-Total phenolic content, TTC-Total tannin content, Standard deviation (SD) (*n* = 3).

Powdered microscopy

The powdered root is light yellow in colour; consist of parenchymatous and sclerenchymatous cells, trachieds and root hairs, medullary rays crossing the parenchymatous cells, starch grains, cork cells with phelloderm (Figure 3).

Physicochemical and phytochemical studies

Physicochemical parameters of *Cleome viscosa* were studied as per the standard protocol of API. These parameters are useful for the standardization and self life determination of the raw drugs. The study reveals that water soluble extractive value was (4.375 %), petroleum ether (0.533 %), methanol extractive value (3.379 %) and hydro-alcoholic value (2.516 %). Total ash, water soluble ash and acid insoluble ash are 6.155 %, 2.376 % and 0.5 % respectively. The value of LOD (loss on drying) was 1.225 % (Figure 4).

Phytochemicals analysis of the *Cleome viscosa* showed that total phenolic content (TPC) is 0.07 % ± 0.0001%, total flavanoid content (TFC) is 0.01% ± 0.0001%, total tannin content (TTC) is 0.05%±0002% and total starch content and total sugar content are 0.35%±0.001% and 0.06%±0.0001% respectively (Figure 2). Phytochemical analysis reveals the presence of phyto-compounds which may be directly or indirectly responsible for the inhibition of alignments.[29]

Quantification of marker compounds

The calibration of HPTLC method was performed using linear range of marker compounds i.e. syringic acid and kaempferol between 200-1200 ng/spot, with regression

Calibration parameters	Syringic acid	Kempferol
Table 1: Calibration parameters of Syringic acid and Kaempferol.		
Range (ng/spot)	200-1200	200-1200
Calibration equation	y = 7E-06x + 0.0001	y = 4E-06x + 0.0003
λ_{max}	278nm	370nm
Regression coefficient (R²)	0.9973	0.9919
Correlation coefficient (r)	0.9986	0.9959
S.D	0.002556	0.00143
Slope	6.8242	03.8085
Intercept	0.00014	0.003273
S.E	0.0001488	0.00014
Average	0.004911	0.00299
Limit of detection (LOD)	0.001236	0.001239
Limit of quantification (LOQ)	0.003745	0.003754

equation (y = 7E-06x + 0.000), (y = 4E-06x + 0.000) and correlation coefficient (r = 0.9986), (r = 0.9959) respectively. In the method, the limit of detection (LOD) and the limit of quantification (LOQ) were found to be 0.001236, 0.003745 and 0.001239, 0.003754 for the compounds syringic acid and kaempferol, respectively (Table 1).

Optimization of HPTLC condition(s), namely, selection of mobile phase, absorption maxima and slit dimensions was standardized to provide an accurate, precise and reproducible method for simultaneous determination of syringic acid and kaempferol. Different combinations of solvent systems were tried based on the chemical nature of targeted compounds and finally, tolune: ethyl acetate: formic acid in the ratio of 7: 3: 0.5 (v/v/v) was selected as the best suited system for efficient separation of these metabolites from other unknown markers. Maximum spectra of syringic acid and kaempferol were obtained at 278 nm and 370 nm after scanning the entire UV range, from 200 to 400 nm. Specificity of the developed method reflects the clear and complete separation of marker(s) peak and correspondingly in sample and standard. The relationship between concentration of marker compound and its corresponding peak area in sample band was investigated (Figure 5 and Figure 6).

Purity of targeted band was checked by comparing the spectra of sample at the start, middle and end position with the band spectra of standard Figure 7). Quantification of syringic acid and kaempferol in the methanolic extracts of *Cleome viscosa* was estimated at the maximum absorption spectrum λ_{max} 278 nm and 370 respectively. Thus, it shows that the planned HPTLC method for simultaneous quantification of syringic acid and kaempferol exhibits excellent compassion. Accuracy, precision (Intra and Inter day), recovery were preferred
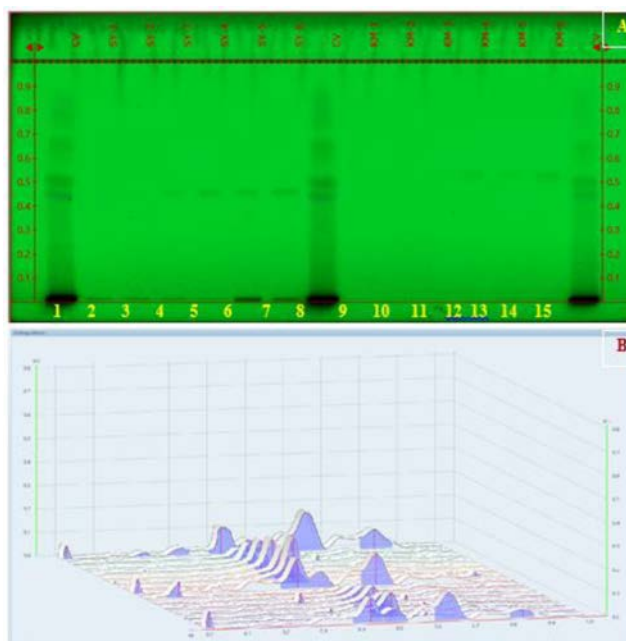
Figure 5: HPTLC fingerprint (A) and 3D densitometric spectra (B) of standards and *Cleome viscosa* (root).

to developed HPTLC method (Table 2 and Table 3). Accuracy of the proposed technique was confirmed by calculating the percentage recoveries of syringic acid and kaempferol in *C. viscosa*. To obtain it, the sample was spiked with three different concentrations and then analyzed by the proposed HPTLC method (Table 4). Quantitative investigation of marker compounds make available a raw data to recognize the effectiveness of sample extracts with compare to pure compounds i.e. syringic acid and kaempferol. Results show that the developed HPTLC method for simultaneous quantification of syringic acid and kaempferol in methanolic extract of *C. viscosa* is accurate, precision, simple, reproducible and specific.

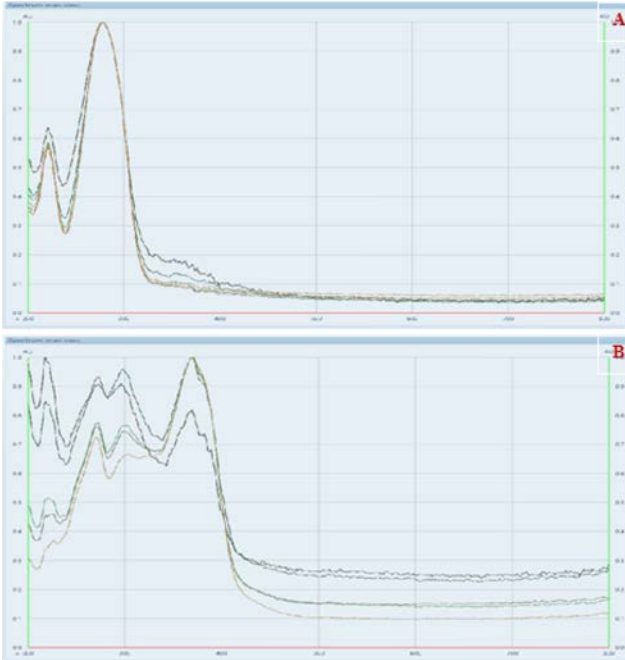**Figure 6: HPTLC chromatogram of Sample (A) and standards (B and C).**

Figure 7: Overlay spectra of syringic acid (A) and Kaempferol (B).

RSD - Relative standard deviation, (*n* = 3)

Table 2: Intra and Inter day Precision Syringic acid.

Con. (ng/ml)	Intra Day			Inter Day								
	Mean AUC	SD	%RSD	Day-1			Day-2			Day-3		
				Mean AUC	SD	%RSD	Mean AUC	SD	%RSD	Mean AUC	SD	%RSD	Mean RSD
200	0.0014	0.0001171	8.21420	0.001563	0.000111	7.074908	0.001517	0.00011	7.26273	0.00142	0.00017	11.8469	8.7281
400	0.0029	0.0001484	5.02606	0.00297	0.000145	4.890855	0.002997	0.000199	6.626639	0.002893	0.00016	5.6298	5.7157
600	0.0045	0.0001588	3.52217	0.004367	0.000159	3.637787	0.00447	0.000207	4.622814	0.004483	0.00013	2.9110	3.7238
800	0.006	0.0001422	2.37270	0.00591	0.000187	3.161005	0.005897	0.00024	4.071274	0.005997	0.00018	3.0032	3.4118
1000	0.0071	0.0001921	2.68783	0.007243	0.0003	4.144806	0.006987	0.000233	3.333213	0.007213	0.00022	3.0509	3.5096
1200	0.0084	0.00013	1.54486	0.008547	0.000135	1.579924	0.00836	0.000156	1.868481	0.008487	0.00017	2.0589	1.8357

Table 3: Intra and Inter day Precision Kaempferol.

Con. (ng/ml)	Intra Day			Inter Day									Mean RSD
				Day-1			Day-2			Day-3			
	Mean AUC	SD	%RSD	Mean AUC	SD	%RSD	Mean AUC	SD	%RSD	Mean AUC	SD	%RSD	
200	0.00096	0.000103	10.65	0.0010	0.00012	12.218	0.00104	0.00012	11.8155	0.00124	0.00011	7.026	10.353
400	0.00197	0.000101	5.118	0.0020	0.00015	7.4017	0.00229	0.00017	7.47002	0.00196	0.00013	6.691	7.187
600	0.00302	0.000103	3.398	0.0031	0.00022	7.2338	0.00297	0.00012	4.21884	0.00356	0.00014	4.051	5.167
800	0.00358	0.000136	3.802	0.0034	0.00010	2.9863	0.00353	0.00035	9.94199	0.00416	0.00011	2.730	5.219
1000	0.00421	0.000123	2.922	0.0044	0.00011	2.6182	0.00432	0.00034	8.04876	0.004833	0.00013	2.619	4.428
1200	0.00502	0.00013	2.592	0.0049	0.00010	2.0420	0.00535	0.00015	2.82884	0.005487	0.00017	3.184	2.685

RSD- Relative standard deviation, (*n* = 3)

Table 4: Recovery studies of Syringic acid and Kaempferol.

Sample	Amount of Standard present in Sample (µg)	Amount of Standard added (µg)	Theoretical added value (µg)	Amount of standard acid analyzed (µg)	Recovery (%)	Average Recovery	SD	Mean RSD (%)
Syringic acid	0.0102	50	50.0102	50.0144	100.00839	100.004	0.00376	0.003766
	0.0102	100	100.0102	100.0112	100.00099			
	0.0102	150	150.0102	150.0154	100.00346			
Kemferol	0.00721	50	50.00721	50.00685	99.999280	99.999	0.000617	0.000617
	0.00721	100	100.00721	100.00765	100.00044			
	0.00721	150	150.00721	150.00755	100.00022			

RSD- Relative standard deviation, (*n* = 3)

Evaluations of biological potential

On the basis of identified marker compounds *in-vitro* biological potential of the *Cleome viscosa* were analyzed. In this, antioxidant activity, anti-inflammatory activity and anti-diabetic analysis were performed.

The ferric reducing power assay of *C. viscosa* methanolic extract served as significant indicator of its oxidant potentiality as reducing agent which in turns signifies its antioxidant activity.[30] In ferric reducing assay, linear equation were found ($y = 0.001x + 0.0415$, $R^2 = 0.896$) which shows the linear increase of antioxidant potential of the species as per the concentration increases. In DPPH method, free radical scavenging extensively used for screening of medicinal plants having antioxidant potential. The scavenging effect of DPPH radical on *C. viscosa* was concentration dependant and varied among samples as well as standards (syringic acid and kaempferol). Data reveals that the IC_{50} value of *C. viscosa* was 149.81± 0.004. Syrigic acid exhibits IC_{50} at 05.331 ± 0.166 μg/ml, which is followed by kaempferol having IC_{50} at 08.583 ± 0.088 μg/ml (Table 5).

In-vitro antidiabetic potential was evaluated by α-amylase inhibition assay based on starch-Iodine and 3, 5 DNS method. In starch-iodine method, data reveals that activity increases linearly with concentration i.e. 0.1- 0.5 mg/ml of *C. viscosa*, IC_{50} value of plant extract 56.2419± 0.266 whilst acarbose exhibit IC_{50} 22.331 ± 0.064 μg/ml. Data of DNS inhibition increases with the concentration, degradation of starch reduces and thus indicating the inhibition of enzyme activity. IC_{50} value of plant extract 88.4211± 0.036 μg/ml whilst acarbose

exhibit IC_{50} 18.003 ± 0.104 μg/ml. Thus, data indicating that *C. viscosa* exhibit the promising antidiabetic potential (Table 6).

All physico-chemical and phytochemical parameters are within the limit of the standard protocol of API, 2005. As per the literature survey and to our knowledge syringic acid and kaemferol is the first time reported in *C. viscosa*. The flavonoids and phenolic content exhibit strong antioxidant potential which depends on the formation of the complex (Ferricyanide convert to ferrous form) with metal atoms i.e. iron and copper. Total phenolic and total flavonoids content was determined. Antioxidants (Syringic Acid and Kaempferol) have a protective effect against the diabeticsby inhibiting free radical generation (peroxidation chain reaction). Therefore, increase in the level of oxygen and nitrogen free radicals linked with lipid peroxidation and oxidation of glucose which contribute towards diabetes control. Similarly, the phenolic compound is shown noteworthy anti-inflammatory potential by inhibiting TNF-α, IL-1β as well as IL-6 mRNA expression.[31] *In-vitro* anti-inflammatory potential of the *C. viscosa* was evaluated by the BSA method,[32] on the basis of identified marker compounds. The analyzed data of *C. viscosa* reveals that IC_{50} value 88.4211± 0.036 μg/ml whilst ascorbic acid exhibit IC_{50} value 04.552 ± 0.008 μg/ml. *In-vitro* biological evaluation of *C. viscosa* reveals significant antioxidant, antidiabetic and especially anti-inflammatory activity. In previous research, no data exist on the availability of identified marker compounds in *C. viscosa* and their correlation with anti-inflammatory activity. Therefore, this study will play a key role in the future for evaluation of the *in-vivo* potential of *C. viscosa* which will a way forward in research on this species.

CONCLUSION

The present study establishes pharmacognostical and phytochemical standards which will be helpful for the identification, standardization and quality evaluation of *C. viscosa* and maintaining batch to batch consistency

Table 5: Yield of Syringic acid and Kaempferol in *Cleome viscose.*

S no	Standards	% yield (mg)
1	Syringic acid	0.0102 ± 0.0003
2	Kaempferol	0.00721 ± 0.0004

SD- Standard deviation, (*n* = 3)

Table 6: *In-vitro* analysis of *Cleome viscosa.*

S/N	Plant/standard	Antioxidant Activity	Anti-diabetic Potential		Anti-inflammatory Potential
		DPPH {IC_{50} (μg/ml)}	DNS Method {IC_{50} (μg/ml)}	Starch- iodine model {IC_{50} (μg/ml)}	BSA method {IC_{50} (μg/ml)}
1	*Cleome viscosa*	149.81± 0.004	88.4211± 0.036	56.2419± 0.266	22.8834± 0.088
2	Syringic acid	05.331 ± 0.166	-	-	-
3	Kaempferol	08.583 ± 0.088	-	-	-
4	Ascorbic acid	-	-	-	04.552 ± 0.008
5	Acarbose	-	18.003 ± 0.104	22.331 ± 0.064	-

RSD- Relative standard deviation, (*n* = 3)

of herbal formulations using this plant as ingredients. HPTLC tool is used in many herbal monographs for the profiling and identification of chemical composition of ayurvedic single drug as well as formulations. Developed HPTLC method is simple, cost-effective and precise for the quantification of syringic acid and kaempferol.
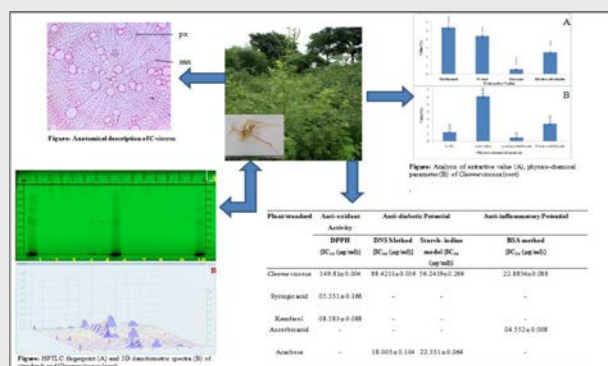
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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Upadhyay RK. *Cleome viscosa* Linn: A natural source of pharmaceuticals and pesticides. Int J Green Pharm (IJGP). 2015;9(2):71-85.

2. Gupta NK, Dixit VK. Evaluation of hepatoprotective activity of *Cleome viscosa* Linn. extract. Indian J Pharmacol. 2009;41(1):36-40. doi: 10.4103/0253-7613.48892, PMID 20177580.

3. Ayyanar M, Ignacimuthu S. Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences. Int J Appl Res Nat Prod. 2009 Sep 15;2(3):29-42.

4. Panduraju T, Parvathi B, Rammohan M, Reddy CS. Wound healing properties of *Cleome viscosa* Linn. Hygeia JD. Med. 2011;3(1):41-5.

5. Mali RG, Mahajan SG, Mehta AA. *In vitro*. Screening of *Cleome viscosa*. Extract for anthelmintic activity. Pharm Biol. 2007 Jan 1;45(10):766-8. doi: 10.1080/13880200701585923.

6. Jayaraman J, Jayaraman J. Laboratory manual in biochemistry. Chichester: Wiley Eastern; 1981.

7. Jente R, Jakupovic J, Olatunji GA. A Cembranoid diterpene from *Cleome viscosa*. Phytochemistry. 1990;29(2):666-7. doi: 10.1016/0031-9422(90)85142-3.

8. Kirtikar K, Basu BD. *Boerhaavia diffusa*. Indian medicinal plants. Lalit Mohan Basu publications. 1956;3(2):45-8.

9. Aparadh VT, Karadge BA. Fatty acid composition of seed oil from some *Cleome viscosa*. Pharm J. 2010 Jun 1;2(10):324-7.

10. Abd El-Gawad AM, El-Amier YA, Bonanomi G. Essential oil composition, antioxidant and allelopathic activities of *Cleome droserifolia* (Forssk.) Delile. Chem Biodivers. 2018 Dec;15(12):e1800392. doi: 10.1002/cbdv.201800392, PMID 30253077.

11. Parimaladevi B, Boominathan R, Mandal SC. Studies on analgesic activity of *Cleome viscosa* in mice. Fitoterapia. 2003 Apr 1;74(3):262-6. doi: 10.1016/s0367-326x(03)00020-0, PMID 12727491.

12. Bawankule DU, Chattopadhyay SK, Pal A, Saxena K, Yadav S, Yadav NP, *et al*. An *in-vivo* study of the immunomodulatory activity of coumarinolignoids from *Cleome viscosa*. Nat Prod Commun. 2007 Sep;2(9). doi: 10.1177/1934578X0700200911, PMID 1934578.

13. Chauhan NS. Medicinal and aromatic plants of Himachal Pradesh. Indus publishing; 1999.

14. Devi BP, Boominathan R, Mandal SC. Evaluation of anti-diarrheal activity of *Cleome viscosa* L. extract in rats. Phytomedicine. 2002 Jan 1;9(8):739-42. doi: 10.1078/094471102321621368, PMID 12587696.

15. Rukmini C. Chemical, nutritional and toxicological evaluation of the seed oil of *Cleome viscosa*. Indian J Med Res. 1978;67:604-7. PMID 680902.

16. Phan NM, Nguyen TP, Le TD, Mai TC, Phong MT, Mai DT. Two new flavonol glycosides from the leaves of *Cleome viscosa* L. Phytochem Lett. 2016 Dec 1;18:10-3. doi: 10.1016/j.phytol.2016.08.020.

17. Brindha P, Niraimathi KL, Karunanithi M. Phytochemical and *in vitro* screening of aerial parts of *Cleome viscosa* Linn. Extracts (Capparidaceae). Int J Pharm Pharm Sci. 2012;4(1):27-0.

18. Anonymous. The ayurvedic pharmacopoeia of India. New Delhi: Government of India, Ministry of Health and Family Welfare; 1989;7-18.

19. Ordonez AA, Gomez JD, Vattuone MA, Lsla M. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem. 2006 Aug 1;97(3):452-8. doi: 10.1016/j.foodchem.2005.05.024.

20. Soni A, Sosa S. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. J Pharm Phytochem. 2013 Nov 1;2(4):22-9.

21. Ayurvedic pharmacopoeia of India. 1st ed: Part 2. Vol. 2; 2009. p. 166-7.

22. Johansen DA. Plant microtechnique. Vol. 530p. New York: McGraw-Hill Book Company, Inc; 1940.

23. Srivastava SK, Chaubey M, Khatoon S, Rawat AKS, Mehrotra S. Pharmacognostic evaluation of *Coleus forskohlii*. Pharm Biol. 2002 Jan 1;40(2):129-34. doi: 10.1076/phbi.40.2.129.5842.

24. Shukla PK, Misra A, Kumar M, Rajan S, Agrawal PK, Rawat AKS, *et al*. Intra-specific chemotypic variability of forskolin content in *Coleus forskohlii* (Wild.) Briq. Growing in Nilgiri Hills of India. JPC - Journal of Planar Chromatography - Modern TLC. 2016 Oct;29(5):347-55. doi: 10.1556/1006.2016.29.5.4.

25. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem. 1987;165(1):215-9. doi: 10.1016/0003-2697(87)90222-3, PMID 3120621.

26. Williams LA, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, *et al*. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Med J. 2008;57(4):327-31. PMID 19566010.

27. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31(3):426-8. doi: 10.1021/ac60147a030.

28. Sonkamble VV, Kamble LH. Antidiabetic potential and identification of phytochemicals from *Tinospora cordifolia*. Am J Phytomed Clin Ther. 2015;3(1):97-110.

29. Anonymous. Official Methods of Analysis of Association of Official Analytical Chemists (AOAC). Virginia; 1984.

30. Jayanthi P, Lalitha P. Determination of the *in vitro* reducing power of the aqueous extract of *Eichhornia crassipes* (Mart.) Solms. J Pharm Res. 2011 Nov;4:4003-5.

31. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines (Basel). 2018 Sep;5(3):93. doi: 10.3390/medicines5030093, PMID 30149600.

32. Anyasor GN, Okanlawon AA, Ogunbiyi B. Evaluation of anti-inflammatory activity of *Justicia secunda* Vahl leaf extract using *in vitro* and *in vivo* inflammation models. Clin Phytosci. 2019 Dec;5(1):1-3.

PICTORIAL ABSTRACT

Figure: Anatomical description of C.viscosa

Figure: Analysis of extractive value (A), physico-chemical parameter(B) of Cleome viscosa(root)

Figure: HPTLC fingerprint (A) and 3D densitometric spectra (B) of standards and Cleome viscosa (root)

SUMMARY

- Phytochemicals are found within the standard limit of Ayurvedic Pharmacopoea of India.
- Identification of phenolic bioactive compounds.
- Validation of HPTLC method for the quantification of pharmacologically active metabolite
- i.e. Kaempferol and syringic acid.
- Promising *in-vitro* antioxidant, anti-diabetic potential and anti-inflammatory potential was
- observed in the *Cleome viscosa*.

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