

Original Article

PHARMACOGNOSTICAL AND PHARMACOLOGICAL EVALUATION OF *CRINUM LATIFOLIUM* L.

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Received: 06 Oct 2017 Revised and Accepted: 26 Sep 2018

ABSTRACT

**Objective:** Pharmacognostical study along with the development of a quantitative HPTLC method for *Crinum latifolium* and evaluation of its traditional claims.

**Methods:** Quantification of three marker compounds oleanolic acid, linoleic acid, and lupeol was done through HPTLC. *In vitro* antioxidant activity was determined by six different models, namely total phenolic and total flavonoid content, DPPH radical scavenging assay, ferric reducing power, antioxidant capacity and hydroxyl radical scavenging assay. *In vitro* antidiabetic activity was evaluated by  $\alpha$ -amylase inhibition assay based on starch iodine and DNS method.

**Results:** The content of oleanolic acid, linoleic acid, and lupeol were found to be higher in aerial parts like 0.015%, 0.048%, and 0.028% respectively, while in root extract 0.006%, 0.027% and 0.025% respectively on a dry weight basis. Free radical scavenging activity was done by DPPH assay, showing the IC<sub>50</sub> value of 410±1.105 µg/ml in roots and 441.95±1.788 in aerial parts. *In vitro* antidiabetic potential of both the parts were assessed by starch iodine color assay and DNS method of alpha-amylase inhibition model. In 3,5 DNS assay, IC<sub>50</sub> of extract from aerial parts was 282.21±2.151µg/ml whereas in root extract it was 193.33±2.45µg/ml. Iodine-starch assay of *C. latifolium* (aerial part) shown the IC<sub>50</sub> value of 340.81±0.49 µg/ml and *C. latifolium* (root) of 74.64±1.28 µg/ml.

**Conclusion:** The results indicate that the aerial parts of the plant possess more antidiabetic potential in comparison to the root. Thus, the aerial part can be used to get better results as a drug and roots can be used as an alternative.

**Keywords:** *Crinum latifolium*, Antioxidant, Antidiabetic, DPPH, HPTLC

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DOI: <http://dx.doi.org/10.22159/ijpps.2018v10i11.22968>

INTRODUCTION

The Indian traditional medicine system is one of the oldest systems of conventional healing. Some of this vast indigenous knowledge is documented in scriptures while a voluminous amount of knowledge is not documented. Such information is localized to some ethnic or rural communities and transferred from one generation to the other only via verbal means. The documented medicine systems include Ayurveda, Yoga, Naturopathy, Unani, Siddha and Homeopathy; abbreviated as AYUSH.

With the recent upsurge of the idea of 'evidence-based validated medicine', scientific evaluation of claims mentioned in ancient texts is now a thrust area of research. This approach has contributed significantly to the scientific validation of the traditional claims.

Amaryllidaceae is a family of perennial, herbaceous, and bulbous plants known for their therapeutic potential in folklore medicine. The genus *Crinum* is widely used in folk medicine and a lot of scientific investigation has been made to explore its pharmacological potential [1]. However, from the literature survey, it was evident that studies related to pharmacognosy of *Crinum latifolium* are relatively less prevalent. The studies of pharmacognostic parameters are equally important to establish the authenticity of a plant as an herbal drug. This formed the rationale behind the present study.

*Crinum*, commonly known as "Sudarshan" in Hindi and "Madhuparnika" in Sanskrit, is used as a tonic, treatment of allergic disorders, inflammation and tumor diseases. The leaf juice is applied topically to skin diseases and on piles to reduce pain and swelling [2]. It is also used as an analgesic, immune stimulating, antineoplastic, antiviral and antimicrobial, as a remedy for blood pressure, rheumatism and in weakness [3]. *C. latifolium* is used in many Ayurvedic formulations such as Mahasudarshanurna which is traditionally used as antiviral, antimalarial and antipyretic [4], thus have huge industrial potential. The

species is rich in glucan A and B, phenylalanine, L-leucin, DL-valin, L-arginin monohydrochloride, latisolin, latisodin, ambellin, 11-o-acetylbambellin, 11-o acetyl 1, 2-β epoxybambellin, crinafolin, crinafolidin, lycorin, epilycorin, epipancrassidin, 9-o-demethylhomolycorin, lycorin-1, o-glucoside, pratorin (hippadin), pratorinin, pratorimin, pratosin, beladin, latindin and latifin [5-6].

To the best of our knowledge, there are few reports available which document the pharmacognostic and pharmacological aspects of *C. latifolium*. Therefore, anatomical descriptors for the plant and a simple, rapid and reproducible HPTLC method for detection and quantification of major compounds such as lupeol, linoleic acid, and oleanolic acid were developed. Further, antioxidant and antidiabetic potential of the root and aerial parts of the *C. latifolium* was studied to evaluate the comparative activity of both the parts.

The objective of this study was to enumerate the pharmacognostic as well as biological activities of root and aerial parts of *C. latifolium*.

MATERIALS AND METHODS

Plant material

Whole plant (aerial part along with roots) of *Crinum latifolium* (fig. 1) was collected in the month of September 2014 from Chitrakoot, Madhya Pradesh (India). The sample was authenticated by Dr. Sharad Srivastava, Scientist; CSIR-National Botanical Research Institute, Lucknow and voucher specimens were deposited in institute's herbarium (LWG no. 262567). Collected sample was washed, shade dried and powdered for further studies.

Chemicals and reagents

Ascorbic acid (>99% w/w), gallic acid (>99% w/w), lupeol (>94% w/w), linoleic acid (>99% w/w), oleanolic acid (>99% w/w), 1-1-diphenyl-2-picrylhydrazyl (DPPH),  $\alpha$ -amylase and 3, 5-Dinitrosalicylic acid was

purchased from Sigma-Aldrich. Other solvents and chemicals (A. R grade) viz. starch soluble, iodine, aluminum chloride, sodium carbonate, Folin's reagent, methanol, ethyl acetate, toluene, formic acid were procured from SD Fine Chemicals, Mumbai, India.

### Anatomical studies

The freshly collected plant material was preserved in 70% ethanolic solution for anatomical studies. The anatomical studies were performed as per standard method [7]. Freehand sectioning was done to obtain thin sections so that cellular details are clearly visible. Sections were stained first with safranin and then with fast green to stain the secondary anatomical structures. The stained sections were mounted with glycerin on the glass slide and then observed under a light microscope. Photomicrographs were taken with Olympus, model CX31 digital microscope at a magnification of 10X for the ocular lens and 10X for the objective lens.

### Preparation of plant extracts

The plant material was manually screened for any impurities and dried in shade, followed by drying in a hot air oven at 40°C and ground (electric grinder) to a fine powder (40 mesh). The powdered sample, 5 gm each of aerial part (Ar.) and root (Rt.) were separately treated with petroleum ether for removal of fatty impurity and then subjected to extraction in methanol. Samples were continuously stirred for 6 h, followed by standing time of 18 h at room temperature and then filtered (Whatman No. 1 filter paper). The extraction process was repeated till complete extraction and the pooled extract was concentrated under vacuum in a rotatory evaporator (Buchi rotavapor, Switzerland) under a standard condition of temperature and pressure. The extract was finally freeze-dried and stored at 4 °C for further use.

### High performance thin layer chromatography

#### Preparation of working solutions

The working solution of standards viz. lupeol, linoleic acid and oleanolic acid (1 mg/ml) and plant samples (10 mg/ml) were freshly prepared with methanol. For calibration, a stock solution of 1 mg/ml each of lupeol, linoleic acid and oleanolic acid were diluted in the same solvent to obtain three working solutions in a concentration ranging from 2-6 µg/ml. The solutions were filtered through a 0.45 µm Millipore membrane filter (Pall, USA) before application.

#### HPTLC conditions

High-performance thin layer chromatography was used for separation of the components present in the extract, both quantitatively as well qualitatively. For quantitative analysis about 10 µl sample was applied using 100 µl sample syringe (Hamilton, Switzerland) on pre-coated plates with silica gel 60F254 of 0.2 mm thickness as 6 mm-wide bands positioned 10 mm from the bottom and 15 mm from side of the plate, using CAMAG Linomat V automated TLC applicator with nitrogen flow providing a delivery speed of 150 ml/s from application syringe. TLC plate developed in a CAMAG twin trough glass chamber which was pre-saturated with mobile phase toluene: ethyl acetate: formic acid (7: 2.5: 0.5 v/v). After the development of the plate, it was dried and then derivatized with anisaldehyde-sulphuric acid and scanned at 580 nm with a TLC scanner (winCATS 1.3.2, CAMAG) [8].

### Pharmacological studies

#### In vitro antioxidant activity

Total flavonoid and phenolic content were estimated [9] and, expressed in terms of mg/g of QE (Quercetin Equivalent) and mg/g GAE (Gallic Acid Equivalent) based on the calibration curve of standard(s) Quercetin and Gallic acid respectively. The radical scavenging potential was analyzed via DPPH radical scavenging assay [10, 11], ferric reducing power [12], antioxidant capacity [13] and hydroxyl radical scavenging [14].

#### In vitro antidiabetic activity (Alpha amylase inhibition assay)

##### Starch-iodine colour assay

The assay was carried out with slight modification based on the starch-iodine test [15]. Methanol extract (500 µL) of varying

concentrations were added to 500 µL of 0.02M sodium phosphate buffer (pH 6.9 containing 6 mmol sodium chloride) containing 0.04 units of α-amylase solution and were incubated at 37 °C for 10 min, then 500 µL soluble starch (1% w/v) was added to each reaction well and again incubated at 37 °C for 15 min 1 M HCl (20 µL) was added to stop the enzymatic reaction, followed by the addition of 100 µl of iodine reagent (5 mmol I<sub>2</sub> and 5 mmol KI). The colour change was noted and the absorbance was read at 620 nm on a microplate reader. The control reaction representing 100% enzyme activity did not contain any plant extract to eliminate the absorbance produced by plant extract; appropriate extract controls without the enzyme were also included. Inhibition of enzyme activity was calculated as:

$$\text{Inhibition}(\%) = \left[ \frac{(C - S)}{C} \right] \times 100$$

Where S is the absorbance of the sample and C is the absorbance of control (no extract).

#### 3, 5-Dinitrosalicylic acid (DNS) method

The inhibition assay was performed using DNS method [16]. Methanol extract (500 µl) of varied concentrations were added to 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mmol sodium chloride) containing 0.04 units of α-amylase solution and were incubated at 37 °C for 10 min, followed by addition of 500 µl of a 1% starch solution (0.02 M sodium phosphate buffer, pH 6.9) in all the test tubes. The reaction was stopped with 1.0 ml of 3, 5 DNS reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. The control sample was also prepared accordingly without any plant extract and were compared with the test samples containing various concentrations of the plant extracts. The results were expressed as % inhibition calculated using the formula.

$$\text{Inhibition} (\%) = \left[ \frac{(C - S)}{C} \right] \times 100$$

where S is the absorbance of the sample and C is the absorbance of control (no extract)

#### Statistical analysis

Results were expressed as mean±SD. Linear regressions analysis was carried out for standards to calculate total phenolic and flavonoid content. IC<sub>50</sub> values were obtained by graph pad prism 5 software. One-way ANOVA followed by student's t test (p<0.01) was used to find the significance of *in vitro* antidiabetic and antioxidant assays.



Fig. 1: *Crinum latifolium* L.

## RESULTS AND DISCUSSION

### Microscopy

*C. latifolium* was studied anatomically to understand the cellular details. Transverse section of the root (fig. 2) shows that the outline is irregularly circular. The epidermis is made up of hexagonal cells followed by multilayered sclerenchymatous cells containing exodermis which is lignified. Cortex is multilayered, cells polygonal in shape. Below the cortex, there is endodermis followed by pericycle. Vascular bundles are arranged in single radii which is exarch and polyarch condition. Treachery element is present in the form of an annular ring. Centrally located pith is present.

The T. S. of the leaf (fig. 3) reveals the presence of single layered epidermis on both upper and lower surfaces followed by a waxy cuticular layer. Cells are more or less circular in shape, but slightly columnar on the adaxial surface of the mid rib region. Stomata are normal with no outer and inner stomatal edges. Mesophyll cells distinguished into two parts, the upper and the lower mesophyll;

upper mesophyll (palisade) is single layered, cells thin-walled columnar in shape, arranged with no intercellular spaces, but with numerous chloroplast; lower mesophyll cells, moderately chlorophyllous, thin-walled, iso-diametric shape and spongy in nature. Concentric vascular bundle of amphicribal type, phloem surrounds the xylem, arranged along the width of the leaf blade.

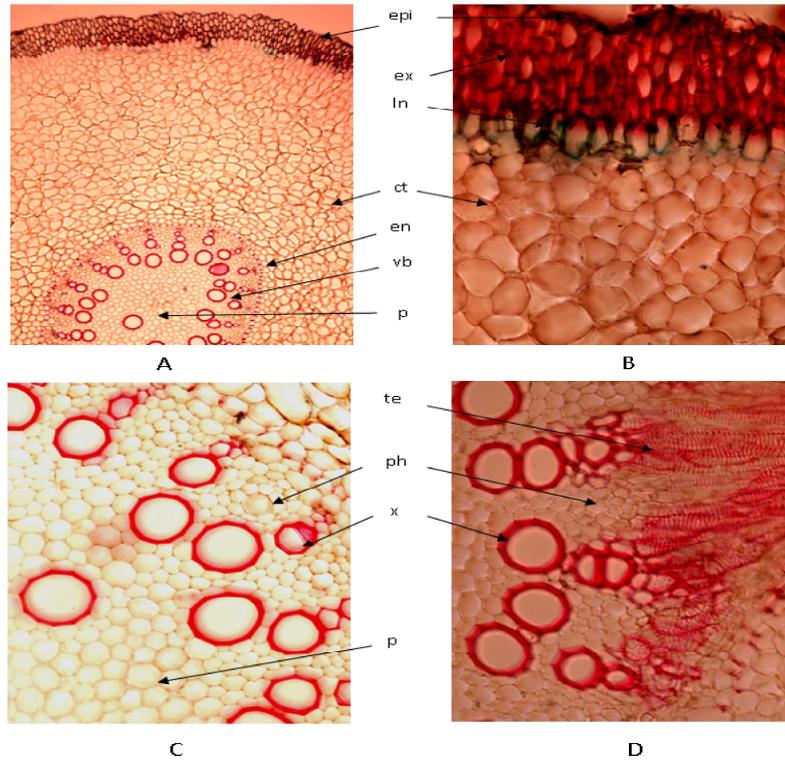


Fig. 2: T. S. of *Crinum latifolium* L. Root A-(10X), B-(40X), C-(40X), D-(40X) Abbreviations: epi-epiblema, ex-exodermis, c-cortex, en-endodermis, vb-vascular bundle, ph-phloem, x-xylem, p-pith, te-tracheary element, ln-lignin

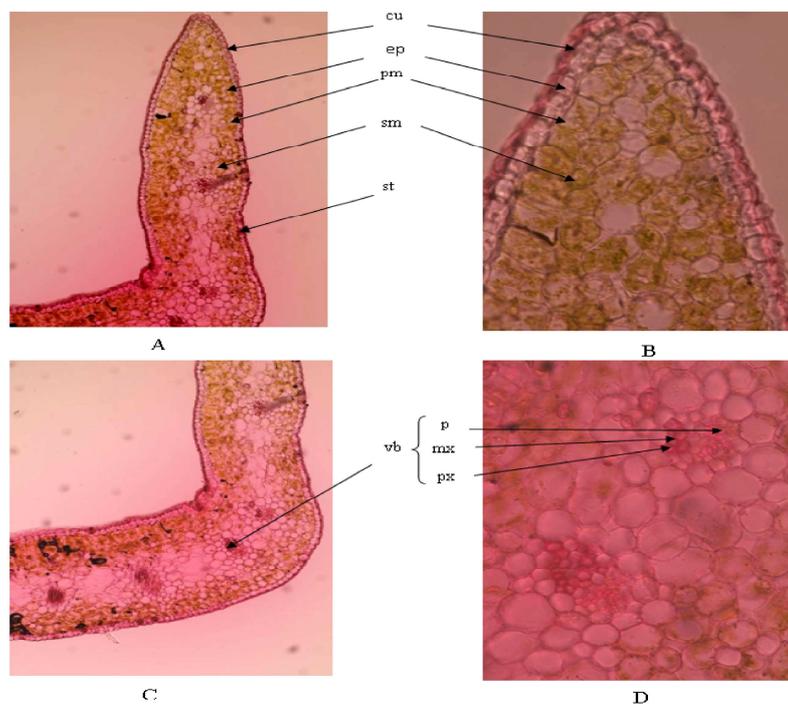
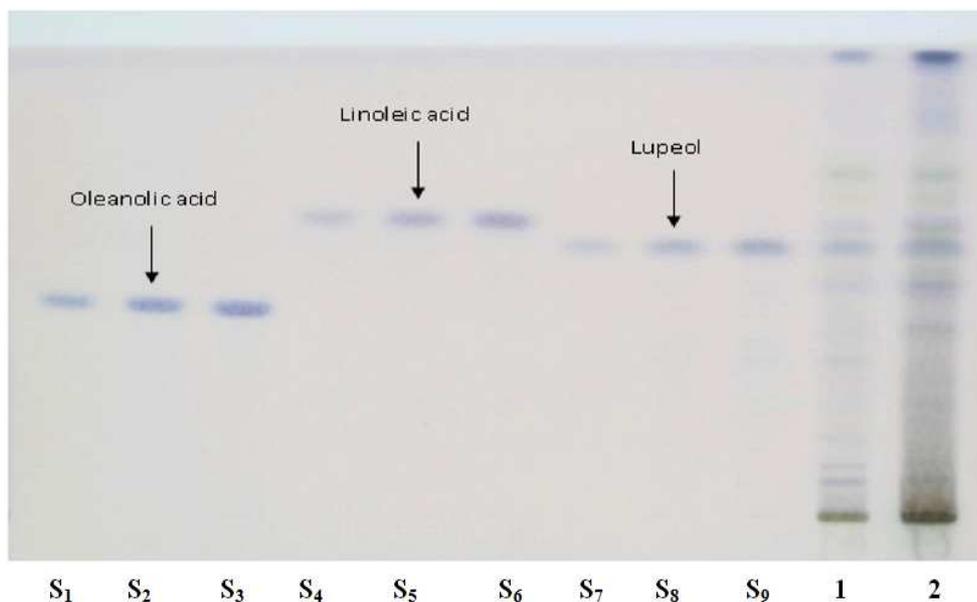


Fig. 3: T. S. of *Crinum latifolium* L. Leaves E-(10X), F-(40X), G-(10X), H-(40X) Abbreviations: cu-cuticle, ep-epidermis, pm-palisade mesophyll, sm-spongy mesophyll, p-phloem, mx-metaxylem, px-protoxylem, vb-vascular bundle, st-stomata

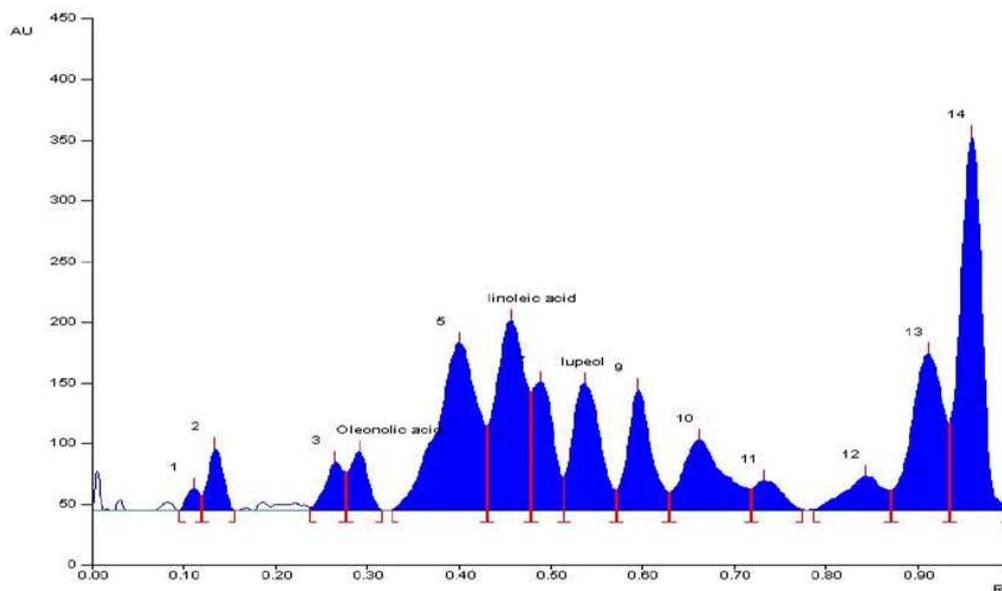
**HPTLC quantification of marker compounds**

HPTLC method was developed for the quantification of targeted marker compounds oleanolic, linoleic acid and lupeol in *C. latifolium*. The extractive yield of *C. latifolium* (Rt.) and *C. latifolium* (Ar.) was 31.0% and 24.0% respectively. TLC images were captured at the visible light after derivatization (fig. 4). The densitometric scan shows the different peaks of the marker compounds in the root (fig. 5) as well as aerial part (fig. 6) samples. Various solvent systems have been tried varying ratio and polarity of constituent solvents of the mobile phase. The mobile phase consisting of toluene: ethyl acetate: formic acid (7: 2.5: 0.5, v/v/v) was optimized for quantitative study. Saturation time of the TLC chamber in the mobile phase was optimized to 20 min for a good resolution of the tested markers and the total run time was 30 min at room temperature

(27±2 °C). The purity of the bands in the samples was confirmed by comparing band spectra of samples with the corresponding band spectra of standards at the start, middle and end position of the bands (fig. 7). Three dilutions of standards were used in the concentration range of 2 to 6 µg/ml and calibration parameters were established (table 1). The maximum concentration of oleanolic acid, linoleic acid and lupeol were found in aerial part i.e., 0.015%, 0.048%, and 0.028% respectively on a dry weight basis, while the root extract contains 0.006%, 0.027%, and 0.025% respectively on a dry weight basis (table 2). Hens developed HPTLC method was found to be simple, accurate, precise and convenient for rapid screening of active constituents present in the methanolic extract of *C. latifolium* and can be used for routine analysis and quality control of herbal material and many formulations containing this plant as an ingredient.



**Fig. 4: HPTLC fig. printing of *Crinum latifolium* L., Abbreviation: S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>-oleanolic acid; S<sub>4</sub>, S<sub>5</sub>, S<sub>6</sub>-linolic acid; S<sub>7</sub>, S<sub>8</sub>, S<sub>9</sub>-lupeol; 1-*Crinum latifolium* (Root), 2-*Crinum latifolium* (aerial part)**



**Fig. 5: HPTLC chromatogram of *Crinum latifolium* (root)**

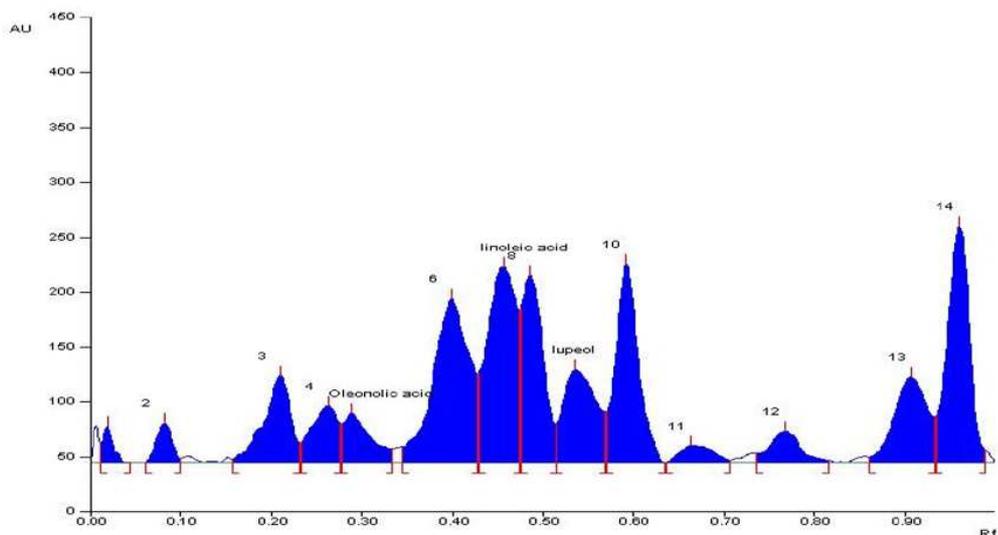


Fig. 6: HPTLC chromatogram of *Crinum Latifolium* (Aerial part)

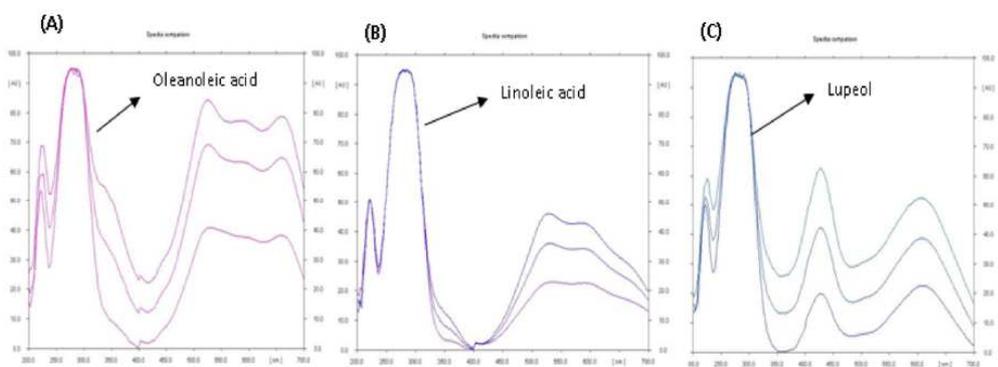


Fig. 7: Purity spectra of (A) oleanolic acid, (B) linoleic acid and (C) lupeol

Table 1: Calibration parameters of marker compounds\*

Parameters	Oleanolic acids	Linoleic acid	Lupeol
Linearity range (µg/ml)	2-6	2-6	2-6
R <sub>r</sub>	0.51	0.75	0.61
Regression coefficient	0.9654	0.982	0.9869
Average	11401.1	8682.55	10710.05
Standard deviation	2948.77	2694.64	3035.18
Standard error	775.67	511.36	492.18
Slop	1448.66	1335.13	1507.58
LOD (µg ml <sup>-1</sup> )	6.72	6.66	6.64
LOQ (µg ml <sup>-1</sup> )	20.35	2018	20.13

\*mean±SD n=3

Table 2: HPTLC quantification of marker compound in *C. latifolium* L

Marker compound	<i>C. latifolium</i> (Rt.)*	<i>C. latifolium</i> (Ar.)*
Oleanolic acid	0.006±0.0025	0.015±0.0026
Linoleic acid	0.0276±0.0012	0.0483±0.0014
Lupeol	0.0256±0.003	0.0287±0.0021

\*mean±SD, n=3; Values on % dry weight basis

**In vitro antioxidant activity**

Polyphenolic compounds are mainly responsible for antioxidant potential of the plants [17]. To assess the antioxidant potential of the plant six models (total phenolic content, total flavonoid content, DPPH radical scavenging assay, Ferric reducing power, Total

antioxidant capacity and 2-Deoxyribose assay) were used. Total phenolic content in the aerial and root extract was found to be 2.32±0.12 mg/g GAE and 3.59±0.25 mg/g GAE respectively while the flavonoid content was reported to be 0.75±0.03 mg/g QE and 0.916±0.011 mg/g QE respectively (table 3).

Table 3: Total phenolic and flavonoid content

Sample	Total phenolic content (mg/g)*GAE	Total flavonoid content (mg/g)*QE
	$y = 186.34x - 0.0119$ $R^2 = 0.9906$	$y = 106.7x - 0.2215$ $R^2 = 0.9922$
<i>C. latifolium</i> (Rt.)	3.59±0.25	0.916±0.011
<i>C. latifolium</i> (Ar.)	2.32±0.125	0.75±0.03

(\*mean±SD, n=3)

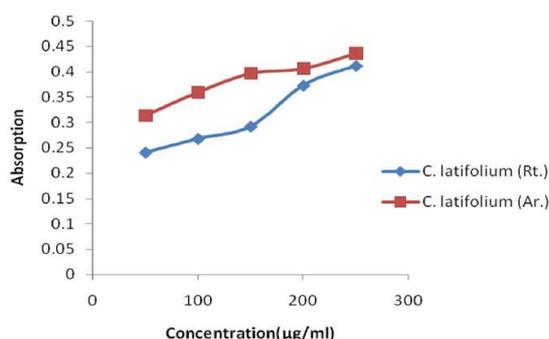
Reducing power activity (fig. 8) of aerial and root extract increase linearly with increase in concentration, with a regression coefficient ( $r^2$ ) of 0.9562 and 0.9532 respectively which is in correlation to the standards i.e. ascorbic acid (0.998), quercetin (0.997) and rutin (0.998). *C. latifolium* showed moderate antioxidant activity against the values of standards (Gallic acid, ascorbic acid, and quercetin) used.

Antioxidant activity of both extracts of the *C. latifolium* was found to increase with the increasing concentration. In DPPH radical scavenging activity, the  $IC_{50}$  value of *C. latifolium* (Rt.) was 410±1.105 µg/ml and *C. latifolium* (Ar.) 441.95±1.788 µg/ml. In hydroxyl radical scavenging, the  $IC_{50}$  value of *C. latifolium* (Rt.) was 50.64±0.869 µg/ml and *C. latifolium* (Ar.) was 158.75±1.394 µg/ml (table 4).

Table 4:  $IC_{50}$  value for *in vitro* anti oxidant activity

S. No.	Plant sample	$IC_{50}$ values (µg/ml)*	
		DPPH assay	2-deoxy ribose
1.	<i>C. latifolium</i> (Rt.)	410±1.105	158.75±1.394
2.	<i>C. latifolium</i> (Ar.)	441.95±1.78	50.64±0.869

(\*mean±SD, n=3)

Fig. 8: Ferric reducing power of *C. latifolium* root and aerial part

#### *In vitro* antidiabetic activity

*In vitro* antidiabetic potential of both the plant parts were assessed by starch iodine color assay and 3, 5 DNS method of alpha-amylase

inhibition model. The starch-Iodine assay reveals that activity increases linearly with concentration i.e. 0.1-0.5 mg/ml of tested plant extract. In 3, 5 DNS assay,  $IC_{50}$  value of root extract and aerial extract was 193.33±2.45 µg/ml and 282.21±2.151 µg/ml respectively whereas in iodine starch assay the values were reported to be 74.64±1.28 µg/ml and 340.81±0.49 mg/ml respectively (table 5) whilst acarbose exhibit  $IC_{50}$  at <25 µg/ml. Results of  $\alpha$ -amylase inhibition by DNS shows that an increase in the concentration of inhibitors, degradation of starch reduces and thus indicating the inhibition of enzyme activity. From the above study, it was observed that the methanolic extract of *C. latifolium* has potential antidiabetic property when compared to the standard drug.

Antioxidant and antidiabetic potential of *C. latifolium* was studied to assess the comparative potential of both the parts. In a nutshell, we concluded that bioactive compounds such as lupeol, linoleic acid, and an oleanolic acid, having well said antidiabetic potential, are present in sufficient quantities in both parts of the plant with promising antioxidant action. From the above study, it is observed that the quantified major bioactive compounds are mainly responsible for the biological potential of the *C. latifolium*.

Table 5:  $IC_{50}$  value for *in vitro* antidiabetic assay

S. No.	Plant sample	$IC_{50}$ values (µg/ml)*	
		DNS method	Starch iodine method
1.	<i>C. latifolium</i> (Rt.)	193.33±2.45	282.21±2.151
2.	<i>C. latifolium</i> (Ar.)	74.64±1.28	340.81±0.49

(\*mean±SD, n=3)

#### CONCLUSION

The present study showed promising anti-diabetic potential in both parts of the plant along with the good antioxidant activity. Therefore, it is necessary to find out the lead compound, which is responsible for the potential of the medicinal plant. Hence, the proposed phytochemical analysis, HPTLC fingerprint, and biological potential can be used as a supportive aid in the quality evaluation and standardization of the raw material used in industries.

#### ACKNOWLEDGEMENT

The authors are thankful to the Director CSIR-NBRI for providing facilities and encouragement throughout the work.

#### AUTHORS CONTRIBUTIONS

PKS, AM, and BK have performed experimental analysis HPTLC, anti oxidant activity, antidiabetic activity. MK has done the botanical

study. RD designed and provided plant material for research. SS design experiments and revised the manuscript writing. All authors read and approved the final manuscript.

#### CONFLICT OF INTERESTS

The authors declare no conflict of interest

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