Study of lycorine

Introduction

The family Amaryllidaceae is a rich source of pharmacologically active constituents like, crinine, galanthamine and Lycorine (John et al. 2012), which are known for their anticholin esterase, anticancerous and expectorant properties. The genus *Crinum* L. have commercial, economical and medicinal importance due to these active constituents. Among four sub families of Amaryllidaceae only Amaryllidoidae contains alkaloids (Hegnauer 1963). The occurrence of a variety of alkaloids has taxonomic significance in Amaryllidaceae. Earlier works recorded 180 types of alkaloids from this family (Lewis 2001, Refaat 2012). Refaat et al. (2002) reported that forty types of Lycorine have been isolated from this genus. The leaf extract of *C.asiaticum* L. has shown anticandidal potential for the presence of lycorine (Surein and Anaya 2014). The leaf extract of *C. latifolium* L. has shown thrombolytic activity (Syed and Das 2013). Chemotaxonomy has been quite successful to delineate three species of this genus as, *C. moorei, C. bulbispernum* and *C. malowanee* .

The active principle lycorine is used for multiple medical purposes, angiogenesis,including anti-tumor, anti-bacteria, anti-virus, anti-inflammation, anti-malaria, inhibition of acetyl cholinesterase, anti-leukemia and anti-cancer, too (Dewan and Das 2013, Jagatap et al. 2014, Miao et al. 2019). It is acolorless crystal and melting point is 260–262 °C and it is a stable compound (John et al. 2012). Previous works have established that Lycorine inhibits cell proliferation and induce cell apoptosis in acute myeloid leukemia (AML) cell line HL-60 (Liu *et al.* 2004), and also an effect on monocyte leukemia cell lineand T-cell leukemia (Evidente et al. 2009). It also blocked multiple myeloma KM3 cell cycle (Li et al.

2007, Evidente et al. 2009, Li et al. 2012). The component also suppressed the growth of various tumor cells – melanoma, ovarian cancer cell, lung cancer and esophageal cancer cells (Lamoral et al. 2009, Liu et al. 2009, Mc Nulty et al. 2009). The present study analyzed Lycorine in different populations, considered as provenances, of *C.asisaticum* L. and *C. latifolium* L. collected from the wild are as of different states.

Materials and Methods

The species *Crinum asiaticum* L. and *Crinum latifolium* L. both have been collected from three different locations as different provenances from different states of India, namely, West Bengal, Assam, Meghalaya and Jammu – Kashmir (Table 1.1 and 1.2).

Study of Biomass

After harvesting, fresh single mature bulbs of both species were weighed and respective dry weight was taken after drying the bulbs in hot air oven at 40 ^{0}C.

Extraction of Lycorine

The fresh 100 g of individual bulbs of both species, cut into small pieces, were crushed into a mortar pestle with 50% glacial acetic acid and kept in a sealed beaker for 72 hours. Thereafter it was filtered through Whatman filter paper no 1 and dissolved in H_2O. Then after acidified with H_2SO_4 to pH 3-4, the crushed material was extracted with petroleum ether and diethyl ether to remove lipophilic, acidic and neutral material. After basifying the aqueous solution to pH 9-10 with NH_4OH, it was extracted with chloroform. The extract was then washed with distilled water to bring back to neutral pH, dried with a rotary evaporator under reduced pressure at 40 °C. The concentrated extract was dried under reduced

pressure to obtain lycorine. The concentrated plant material dissolved in 80% methanol for further study.

Quantitative estimation of Lycorine

The extract was quantified by High Performance Liquid Chromatography (HPLC). The analysis of lycorine was done through HPLC (Agilent) with UV detector. Crude fine extract (10 mg) was dissolved in methanol (1 mL) and injected 20 µL to high performance liquid chromatography (HPLC) using C18 column, eluted with CH_3OH : H_2O (95:5) at flow rate 1.0 ml/min and detected under UV (at 340 nm). Quantitative determination was carried out by the external standard method based on peak area (Kim et al, 2008).

Percentage of lycorine

Percentage of lycorine was calculated by getting the weight of lycorine from initial 10 g of bulb powder. Then it was calibrated in terms of the weight of lycorine in 100 g of bulb powder.

$$Lycorine(\%) = \frac{\text{Lycorine present in 10 g of tissue}}{\text{Initial weight of tissue (10 g)}} \times 100$$

The amount of total lycorine available in each species, obtained after each treatment, has been measured and expressed as the dry weight of each sample.

Average of total lycorine (gm) = dry weight (gm) of specific treated bulbs × percentage of lycorine obtained from that respective species.

Statistical analysis of experimental data

The experimental data were statistically analyzed with SPSS software. The level of significance used in F test was P = 0.05. The means followed by the same lower case letters do not differ significantly at the 0.05 probability level.

Result

Two different species have shown the presence of different percentages of active principle (Table 6.3 and 6.4). Out of two species *C. latifoliunm* contains greater amount of lycorine 0.095 and 1.205 g (Table 6.6). *C. latifolium* has also shown greater amount of biomass 12.68 g (Table 6.2).

Table 6.1: Biomass study of *C. asiaticum* L.

Provenances	Fresh weight(g)	Dry weight(g)
CAKO	7.31	0.79
CAMO	24.50	2.32
CANA	82.36	10.39
CANO	30.65	2.98
CAPA	52.39	5.26
CAPU	33.96	4.96
CASH	30.26	3.25
CASU	8.69	0.78

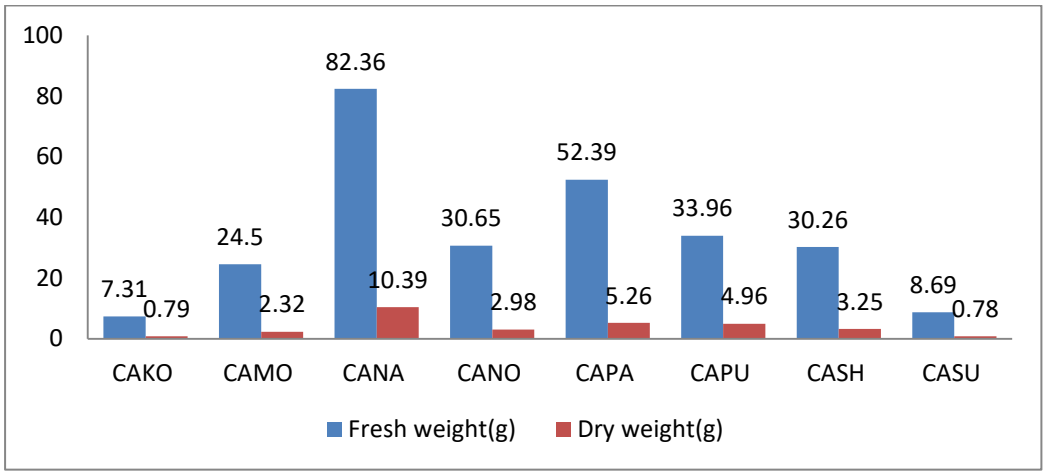

Figure 6.1: Comparative graphical presentation of biomass of *C. asiaticum*.

Table 6.2: Biomass study of *C.latifolium* L.

Provenances	Fresh weight(g)	Dry weight(g)
CLAS	79.00	10.84
CLBA	33.9	4.84
CLGA	50.79	7.83
CLKA	50.25	7.19
CLKO	30.30	3.12
CLNA	120.28	12.13
CLOD	52.45	8.63
CLPA	89.41	12.68
CLPU	52.50	7.40
CLSH	53.25	7.73

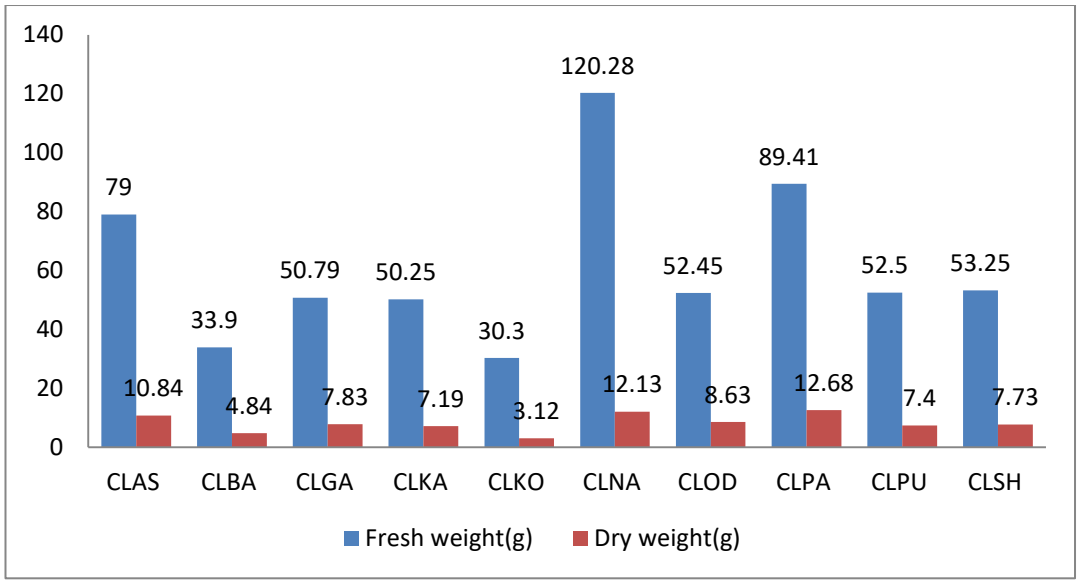

Figure 6.2: Comparative graphical presentation of biomass of *C. latifolium*.

Table 6.3: Percentage of lycorine in different provenances of *C. asiaticum* L.

Name of provenance	Lycorine (%)
CAKO	0.035
CAMO	0.011
CANA	0.120
CANO	0.027
CAPA	0.065
CAPU	0.065
CASH	0.049
CASU	0.029

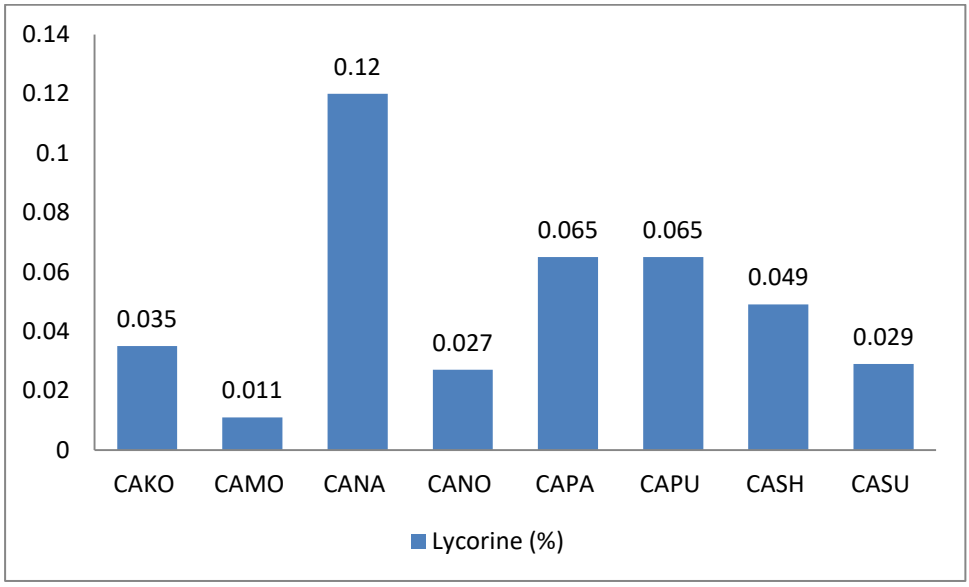

Figure 6.3: Graphical presentation of percentage of Lycorine in different provenance of *C. asiaticum*.

Table 6.4: Percentage of lycorine in different provenances of *Crinum latifolium* L.

Name of provenance	Lycorine (%)
CLAS	0.090
CLBA	0.006
CLGA	0.014
CLKA	0.002
CLKO	0.150
CLNA	0.047
CLOD	0.100
CLPA	0.095
CLPU	0.010
CLSH	0.016

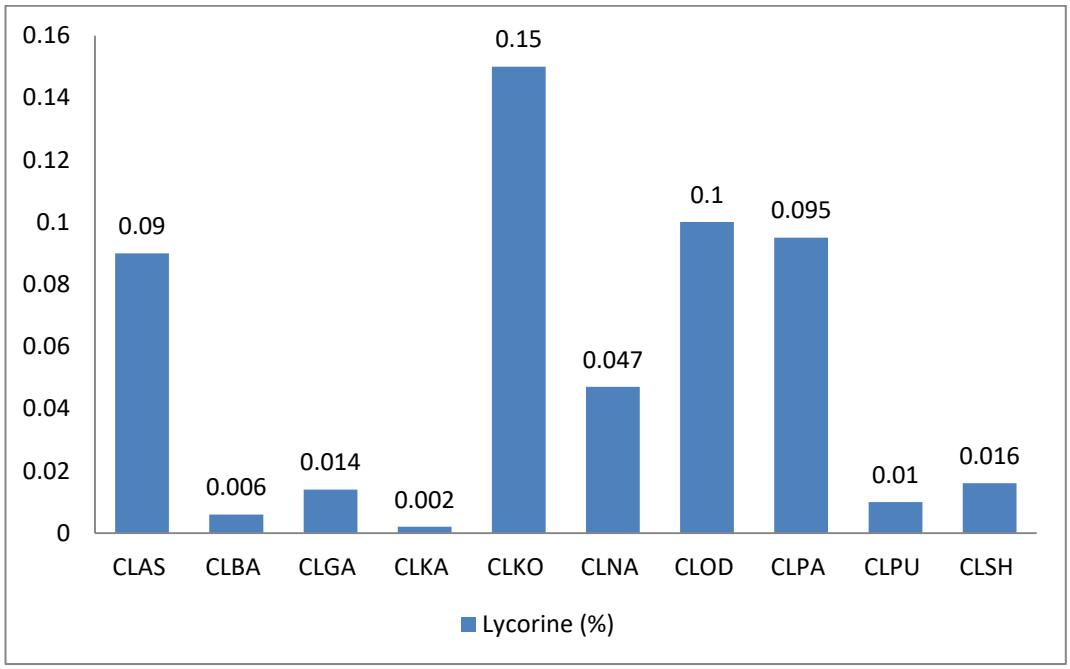

Figure 6.4: Graphical presentation of percentage of Lycorine in different provenance of *C. latifolium*.

Table 6.5: Amount of lycorine in different provenances of *C. asiaticum* L.

Name of provenance	Amount of lycorine (g)
CAKO	0.028
CAMO	0.025
CANA	1.247
CANO	0.080
CAPA	0.342
CAPU	0.322
CASH	0.159
CASU	0.023

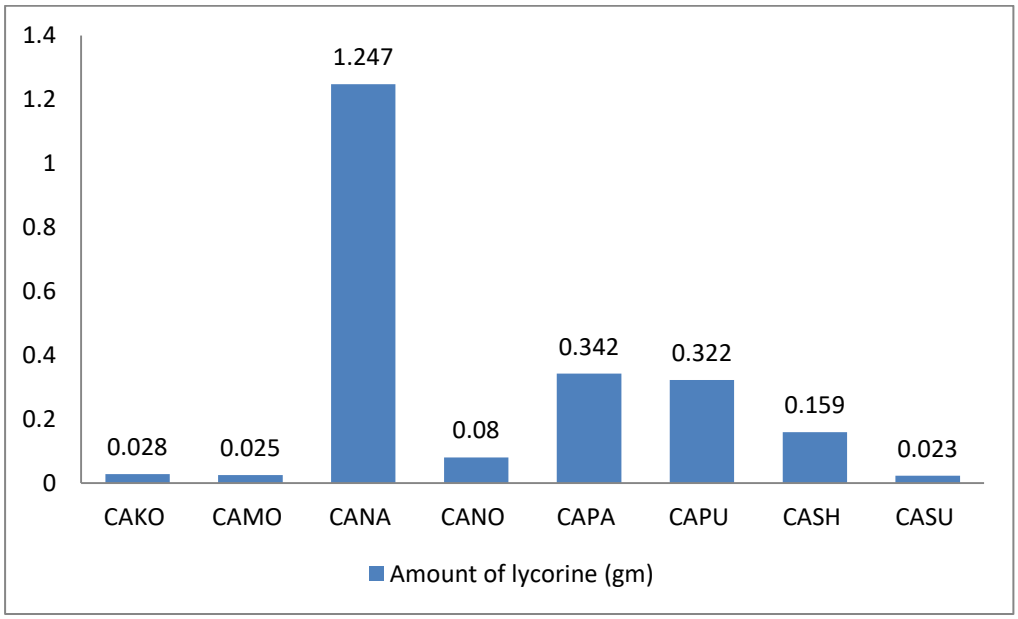

Figure 6.5: Graphical presentation of amount of lycorine in different provenance of *C. asiaticum*.

Table 6.6:Amount of lycorine in different provenance of *C. latifolium* L.

Name of provenance	Amount of lycorine (g)
CLAS	0.976
CLBA	0.029
CLGA	0.101
CLKA	0.014
CLKO	0.468
CLNA	0.570
CLOD	0.863
CLPA	1.205
CLPU	0.074
CLSH	0.124

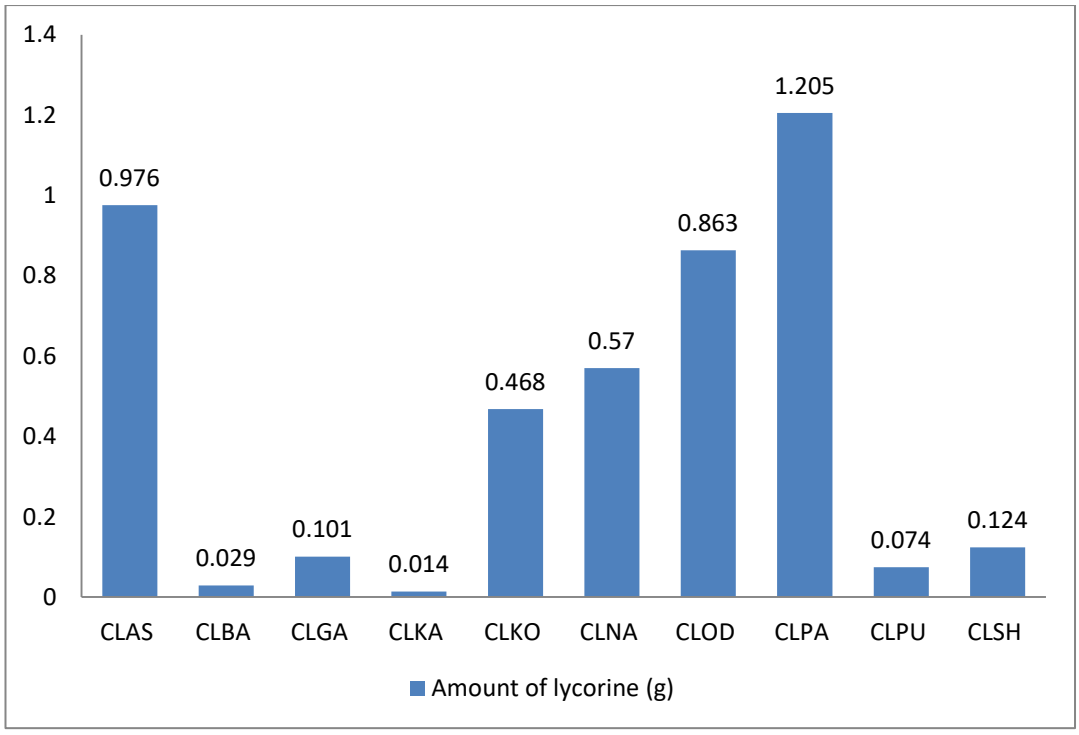

Figure 6.6: Graphical presentation of amount of lycorine in different provenance of *C. latifolium*.

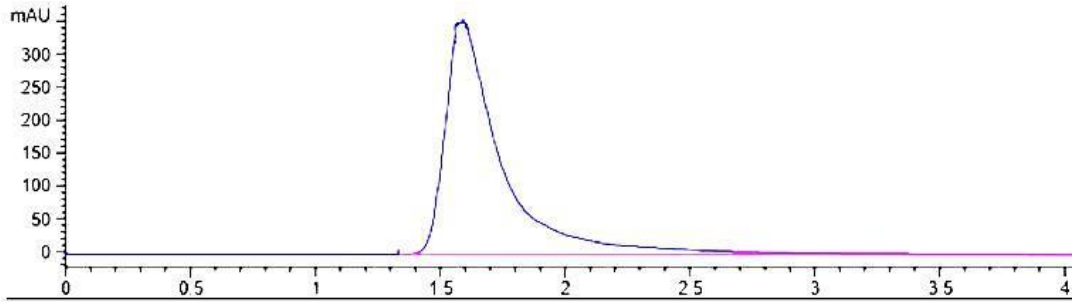

Figure 6.7: Hplc graph of standard lycorine

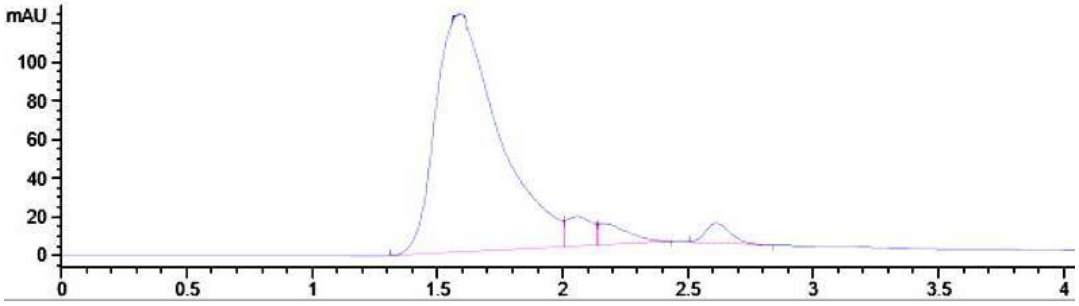

Figure 6.8: Hplc graph of *C. asiaticum* Kolkata provenance.

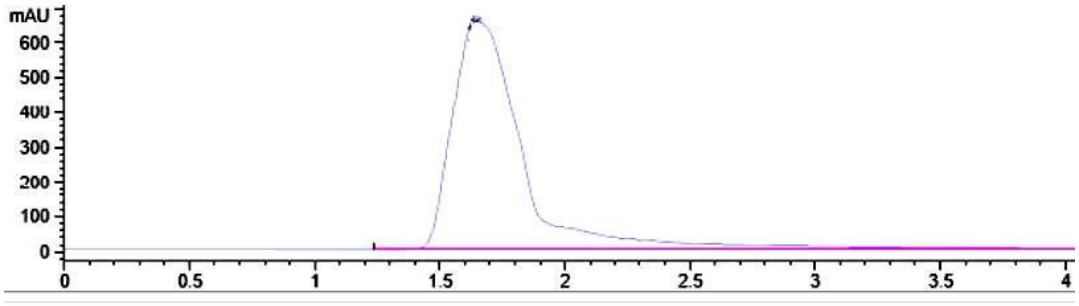

Figure 6.9: Hplc graph of *C. asiaticum* Mongpoo provenance.

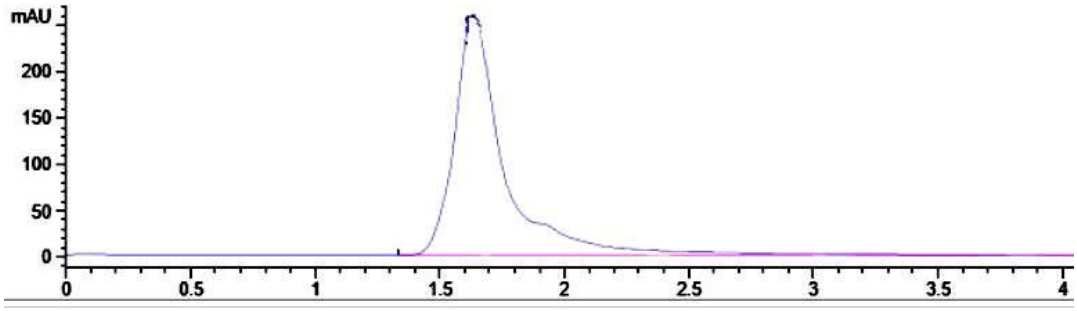

Figure 6.10: Hplc graph of *C. asiaticum* Nadia provenance.

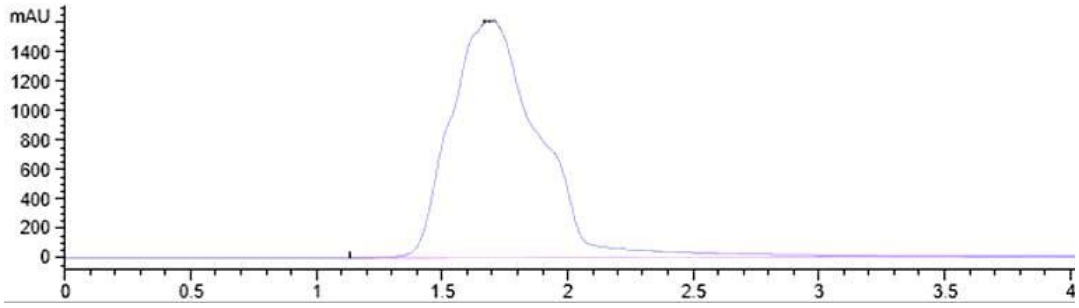

Figure 6.11: Hplc graph of *C. asiaticum* North 24 pargana provenance.

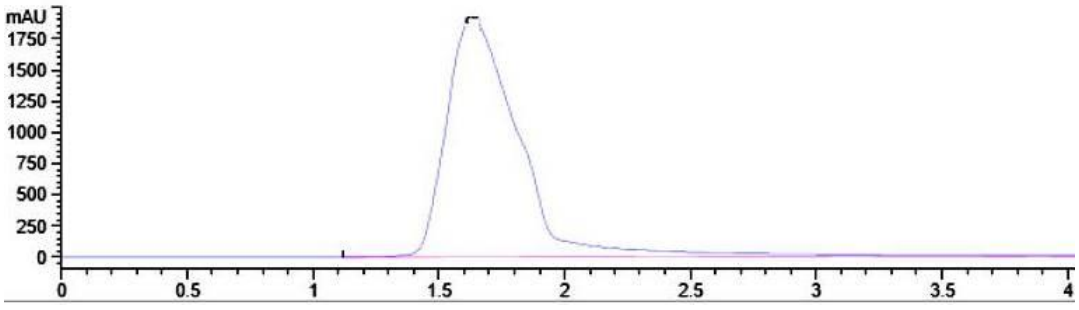

Figure 6.12: Hplc graph of *C. asiaticum* Paschim Medinipur provenance.

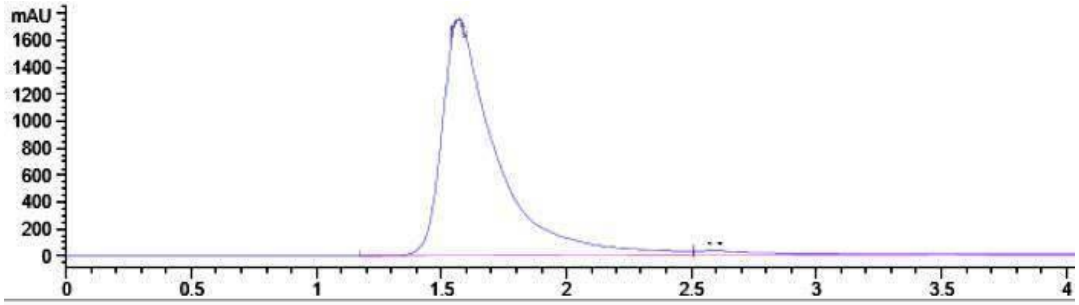

Figure 6.13: Hplc graph of *C. asiaticum* Purba Medinipur provenance.

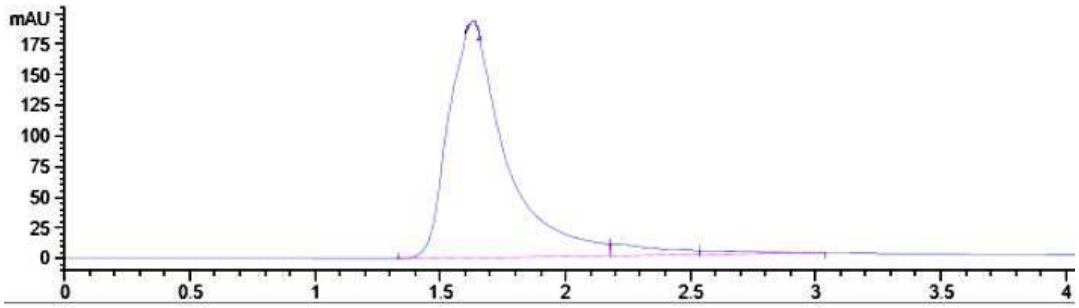

Figure 6.14: Hplc graph of *C. asiaticum* Shillong provenance.

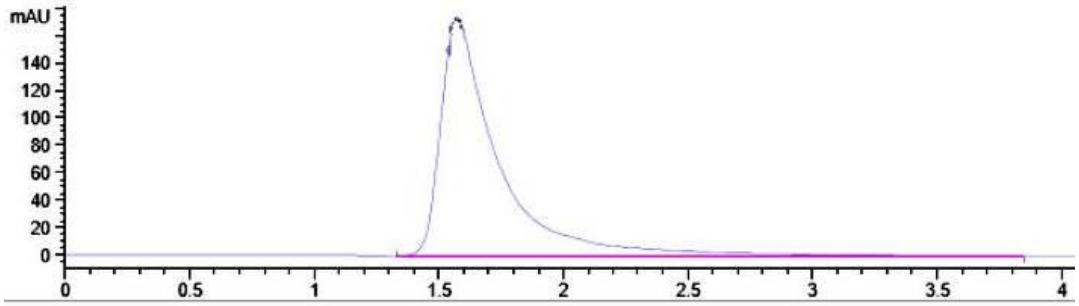

Figure 6.15: Hplc graph of *C. asiaticum* Sundarban provenance.

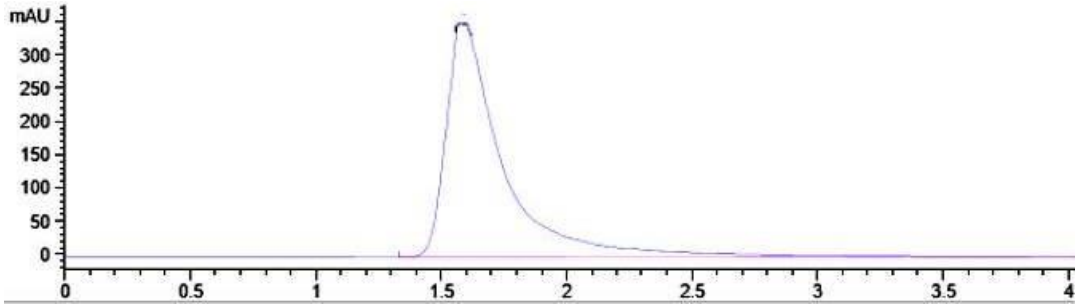

Figure 6.16: Hplc graph of *C. latifolium* Assam provenance.

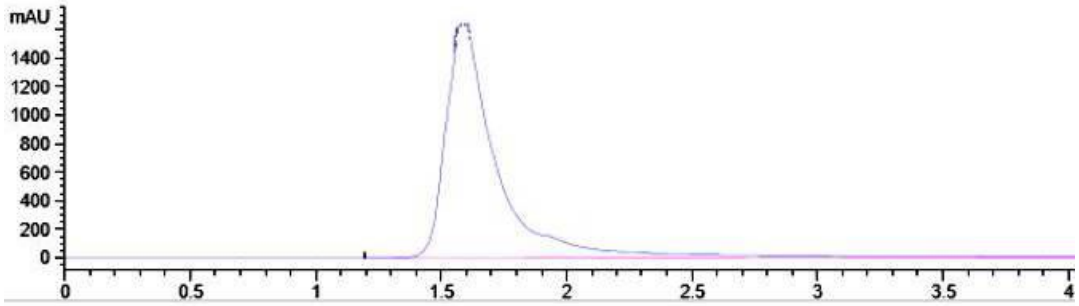

Figure 6.17: Hplc graph of *C. latifolium* Bankura provenance.

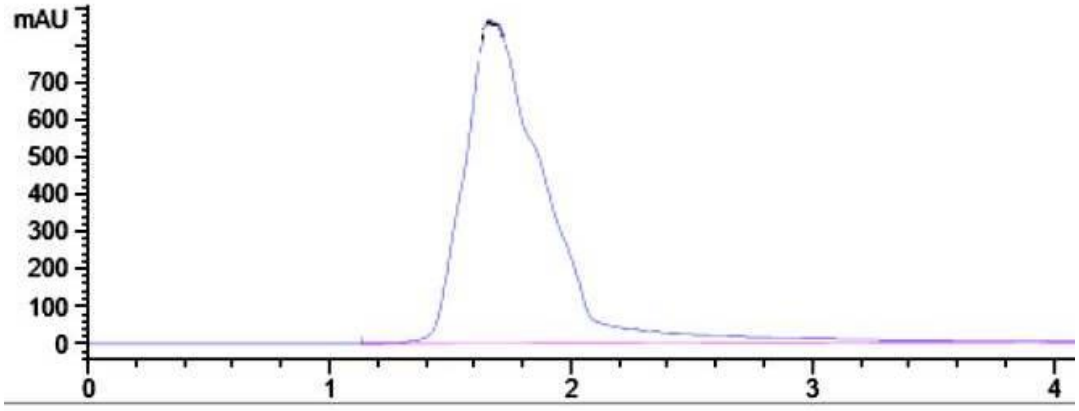

Figure 6.18: Hplc graph of *C. latifolium* Gangtok provenance.

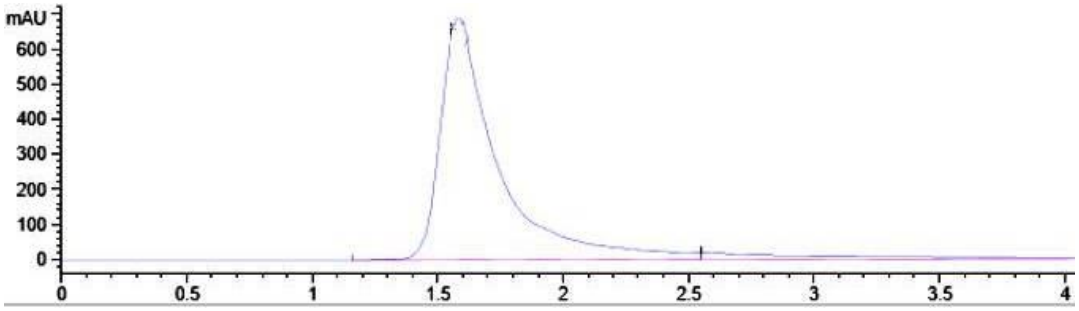

Figure 6.19: Hplc graph of *C. latifolium* Kashmir provenance.

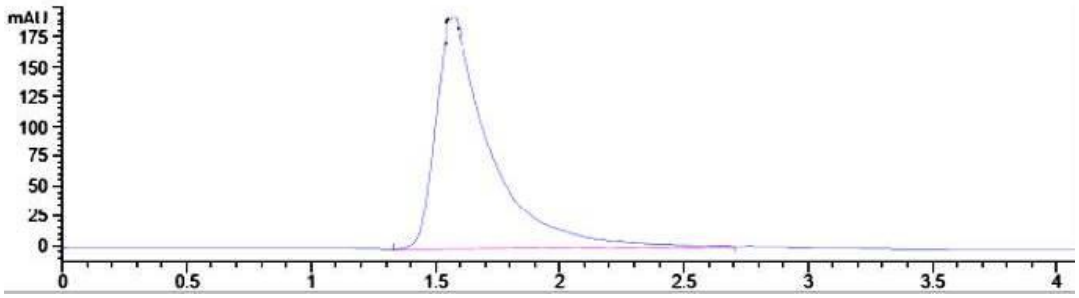

Figure 6.20: Hplc graph of *C. latifolium* Kolkata provenance.

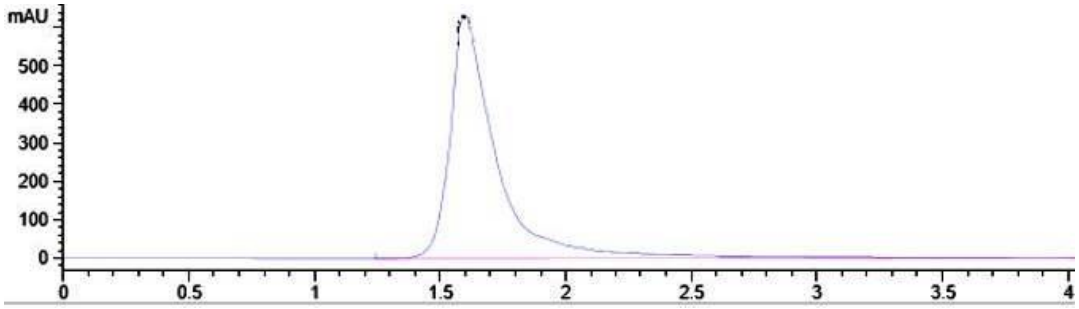

Figure 6.21: Hplc graph of *C. latifolium* Nadia provenance.

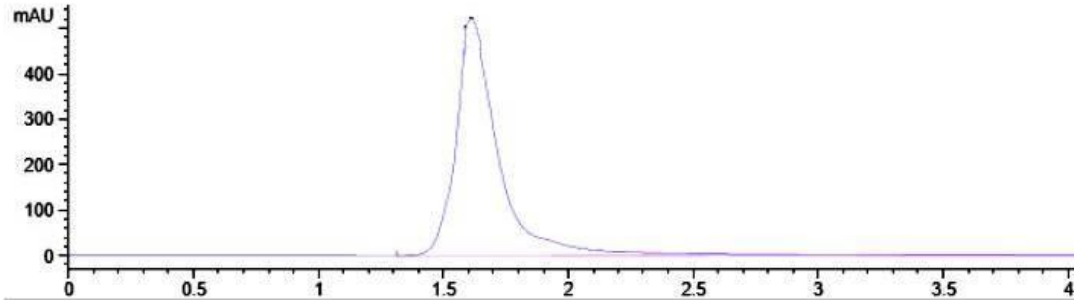

Figure 6.22: Hplc graph of *C. latifolium* Odisha provenance.

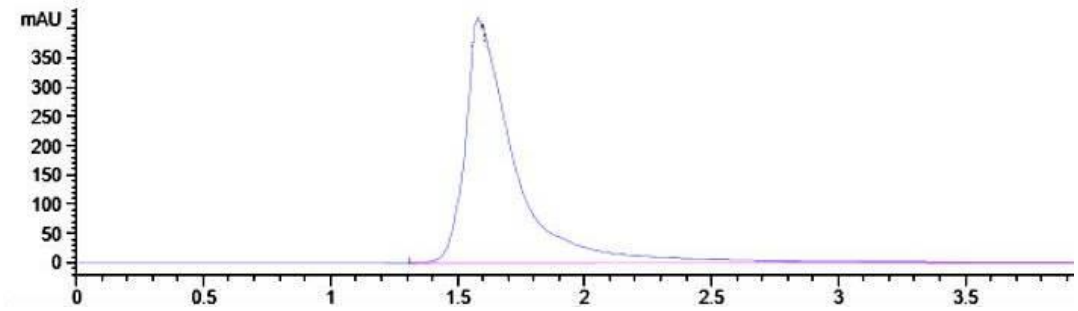

Figure 6.23: Hplc graph of *C. latifolium* Paschim Medinipur provenance.

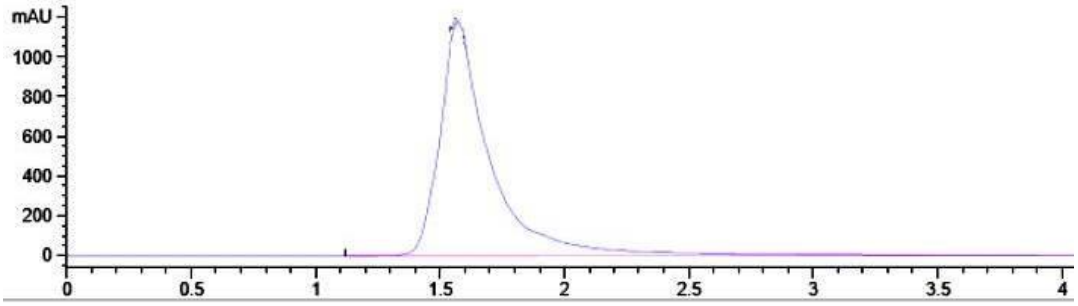

Figure 6.24: Hplc graph of *C. latifolium* Purba Medinipur provenance.

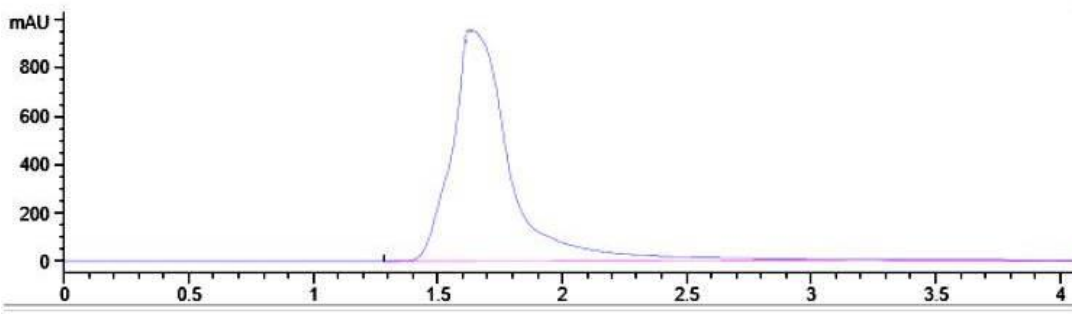

Figure 6.25: Hplc graph of *C. latifolium* Shillong provenance.

Discussion

Lycorine is an important bioactive constituent has been extracted and estimated with the help of HPLC among the studied populations. Lycorine is the main active principle present in both the species *Crinum asiaticum* L. and *Crinum latifolium* L. The presence of this medicinally important biomolecule has been reported by earlier workers for the both of the species (Ghosal et al. 1983, 1989). In this study it is reported that availability of Lycorine in the locally available individual of the species and measure the relative amount among the provenances. The biomass study among the eight different population of *Crinum asiaticum* L. has shown different amount among the populations. *Crinum asiaticum* L. of Nadia has been contain the highest amount of biomass, whether *Crinum asiaticum* L. collected from Sundarban are shown the lowest amount (0.023 gm) (Table 6.5).

On the other hand, after studies of ten populations of the species *Crinum Latifolium* L. of Paschim Medinipur having the highest content of biomass (Table 6.2). The biomass study of both the species has been noted that *C. latifolium* L. of different localities have contained more weight than the *Crinum asiaticum* L. Though, the morphological appearance did not support of this view, it is noted that *Crinum asiaticum* L. have shown the giant figure. In early have no such reports of biomass study for both the species till date.

The percentage of lycorine in among the population of *Crinum asiaticum* L. collection from Nadia of West Bengal shown the highest result (0.120%) (Table 6.3). In other case, ten populations of *Crinum latifolium* L. have shown higher percentage of lycorine is being present in Kolkata population. Whether, the amount of lycorine of Paschim Medinipurh as shown the highest amount of lycorine

contained, due to its more amount of biomass than other populations. The presence of lycorine in the both species it is noted that *Crinum latifolium* L. have contained more amount than *Crinum asiaticum* L. Different alkaloid extracted from various species of Amaryllidaceae shows diverse activity like antitumor, anti-bacterial, anti-malarial, anti-viral, anti-cancer and also inhibitor of the Alzheimer disease (Zogh 2010, Asma et al. 2011, Refeat et al. 2012, Jagatap et al. 2014). Earlier reports support the view of presence of most bioactive lycorine in *C. latifolium* L. compare to *Crinum asiaticum* L. The identity of the alkaloid content of a plant not only has important chemical implement but also be implied to demarked taxonomic critical. The global demand for natural active compounds to face the day after day challenging diseases by searching for prospective active chemicals from natural sources, species of *Crinum* L. is an essential source of different bio active alkaloids. The genus has contained more than 180 types of different medicinally important alkaloids (Refeat et al. 2012, Endo et al. 2019).

Conclusion

To meet the global demand of active component the study helps to identify better sources of lycorine among the eighteen populations. The present study investigates the better production of lycorine between two types also. Here it is noted that between two species *Crinum latifolium* is more effective to produce lycorine. The active component lycorine is differentiate all the studied populations among them the accession from Nadia of *Crinum asiaticum* L. is contain highest amount of lycorine on the other hand accession from Paschim Medinipur of *Crinum latifolium* L. is showing the highest amount of lycorine.