**International Journal of Botany Studies**
**ISSN: 2455-541X; Impact Factor: RJIF 5.12**
**Received: 03-07-2020; Accepted: 18-07-2020: Published: 04-08-2020**
**www.botanyjournals.com**
**Volume 5; Issue 4; 2020; Page No. 170-175**

# Phytochemical analysis and Colchicine concentration in cultivated *Gloriosa superba* L among different accessions of Tamil Nadu State, South India

**JA Paul Jasmine[1], V Gurusamy[2], K Palanisamy[3], V Balakrishnan[4], T Sundari[5]**
[1] Research and Development Center, Bharathiar University, Coimbatore, Tamil Nadu, India
[2] PG and Research Department of Botany, H.H. Rajah's College, Pudukkottai, Tamil Nadu, India
[3, 4] PG and Research Department of Botany, Arignar Anna Government Arts College, Sanyasikaradu, Namakkal, Tamil Nadu, India
[5] Department of Chemistry, K.S.R. College of Engineering, Tiruchengode, Tamil Nadu, India

**Abstract**
*Gloriosa superba* L is an important and cultivated medicinal plant. The seed and tubers are containing alkaloids such as Colchicine and Colchicoside. In the present investigations, the phyto-compounds from the different accessions of tubers of *Gloriosa superba* L cultivars from Sirumalai (GA1) region, Mulanoor (GA2)region, Thuraiyur (GA3)region, Konganapuram (GA4) region and Vedaranyan (GA5) region were extracted by using ethanolic extract and the chemical composition and its concentration in the tubers of *Gloriosa superba* were determined by the method of HPLC analysis. The methanolic extract is used for to find out various phytochemical constituents. The five different accessions of different agro climatic zone cultivated *Gloriosa superba* L selected for present investigation. The result shows that Colchicine concentration in all five different accessions.

**Keywords:** colchicine, *Gloriosa Superba*, accessions, alkaloid

## 1. Introduction
Geology is a selective force on plant life in various ways (Krucheberg, 1986) [12]. Soil formation is involved in plant differentiation and provided a valuable insight to the role of material selection in evaluation (Reddy *et al*., 2012) [24]. *Gloriosa superba* L is an important medicinal plant and cryptophytes with ephemeral, elimtring sterry with a perennial sub terranean hypopodial tubers (Le Rocek and Robbertise, 1994 a) [14].The alkaloid Colchicine is reported one of the 19-Tropolent alkaloids and extracted from the tubers and seeds of *Gloriosa Superba* L (Dvoracova *et al*., 1984)[8].Colchicine is a poisonous alkaloid used for western orthodox medicine for the relief of pain and inflammation (Hutchings and Erblanche, 1989) [11].Chemical analysis shows that the presence of Colchicine in *Gloriosa suerba* L (Sarin *et al*. 1974) [25].*Gloriosa superba* L is a angiosperm species in angiosperm plant group and belongs to the family Colchicaceae.

The plant native is Africa and Asia. It is one of the important ornamental plant across the world. The plant is commonly used as medicine, poisonous plant as a noxious weed (Raja Naika *et al*., 2015 [23]; Paul Jasmine and Balakrishnan, 2018) [19]. Each and every part of plant part is poisonous specifically the tuberous rhizomes. Narayanaswamy *et al*. (2014) [16] reported that the plant also contains an alkaloid Gloriocive. Over the years, colchicine resistance has been defined in different ways. The colchicine resistance has evolved along with various features to invent the new drug because of alkaloid.

Importantly, none of the children developed secondary amyloidosis while receiving alkaloid colchicine treatment. Growth, development and subsequent fertility were judged to be normal (Seza *et al*., 2017 [27]; Paul Jasmine *et al*., 2020a&b) [20, 21].

Colchicine also prevents microtubule assemblages and there by disrupts generation of leukotrienes, inflammasome activation, microtubule-based inflammatory cell chemotaxis and cytokines, and phagocytosis. Many of the cellular processes can be identified in other diseases involving in chronic inflammation. The mechanism of action of colchicine alkaloids suggests that potential efficacy of colchicine in other comorbid conditions in associated with gout, such as osteoarthritis and cardiovascular diseases (Nicola *et al*., 2014 [17]; Datta *et al*., 2005) [5].

Colchicine is an important alkaloid obtained from *G.superba*, which is used during the cell division binds to tubulin protein of the spindle fiber and stops the formation of microtubules (Molad, 2002) [15]. During the of meiosis process, it prevents separation of chromatids (Tambong *et al*., 1998) [34] inhibits cytokinesis (Antoccia *et al*., 1993) [1] and induced inter meiocyte connections in *Helianthus annuus* Gautam and Kumar (2013) [9]. The present study deals with the phytochemical analysis and concentration Colchicine of five different accessions of cultivated *Gloriosa superrba* L in Tamil Nadu state.

## 2. Materials and Methods
### 2.1 Plant collection
The five ecotypes of *Gloriosa superba* L cultivated in the places such as Sirumalai (GA1), Mulanoor (GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyam (GA5) belongs to the districts such as Dindigul, Tiruppur, Trichy, Salem and Nagapatinam respectively. The structure of Colchicine are provided (Fig.1) (Table.1). They were indentified and authenticated by the Botanical Survey of India, TNAU (Southern Circle), Coimbatore, Tamil Nadu, India. The plants were deposited in the PG and Research

Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India.

**2.2 Cultivation of plant species in the field**
Five accessions from different regions of Tamil Nadu state selected for the present investigation. The Randomized Block Design (RBD) with three replication was done. The experimental plot mixed with red soil, sand, vermicomost and farmyard manure applied in the ratio of 1:1:1:1 and to enhance macro and micro nutrients in the soil for better tuber growth of *Gloriosa superba*. The cultivated plots were irrigated periodically during the cultivation. Meantime the recommended agronomic and plant protection methods were followed.

**2.3 Harvest of tubers**
The fresh tubers of Gloriosa superba were collected from the five different accessions of Tamil Nadu state. The tubers were dried with the help of sunlight and minimum five days. The collected tubers were generally used for extraction of Colchicine content.

**2.4 Preparation of tuber extract**
500 mg of dried tubers were taken and powdered. The powdered materials of five different accessions were macerated with 25ml of methanol and kept in room temperature at least 24 hours. Then the sample was sonicated up to 45 minutes in an ultrosouicator. Finally, the methanolic extract was filtered and make up a final volume of 25 ml with methanol. The extract was filtered through 0.22 Micron filter before the HPLC analysis (Daniel *et al*., 2003) [4].

**2.5 Qualitative estimation**
The five different accessions of Gloriosa superba L underwent for quantitative estimation was carried out through RP-HPLC method using a C18 Precolumn packed with Kromasil. Water and acetonitrile generally used as mobile phase in the ratio of (70:30) (Chitra and Rajaman, 2009) [3]. Hewlett – Packard series 1050 liquid chromatograph was equipped with a quaternary pump system, diode act as a detector operating at 350 nanometer. Data process module was generally used for the purpose of analysis (Banerjee and Kalloo, 1987) [2].

**2.6 Detection of alkaloid using TLC**
Silica gel was used as stationary phase. Methanol and chloroform (70:30) was a solvent system and used in mobile phase. Dark brown spots were obtained by spraying Dragenroffs reagent in the band obtained from methanol extracts of tubers of Glorioa superba L.

**2.7 Alkaloids**
About 0.2gram of the tuber extracts was wormed with 2 percent H2SO4 for two minutes. It was filtered and a couple of drops of Dragondorff reagent were also added. Orange red colour precipitate indicated the presence of alkaloids.

**2.8 Flavonoids**
Four ml of Gloriosa tuber extract solution was treated with 1.5 ml of fifty percent methanolic solution. The solution was warm in condition and metal magnesium was also added. To this solution, 5 to 6 drops of Conc. HCl was added and then red colour was appeared for flavonoids content and orange colour was also indicates that the presence of flavones.

**2.9 Cardiac glycosides**
25ml of diluted sulphuric acid was added to 5ml of tuber extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH and then five ml of Fehling solution added. Glycosides are indicated by a brick red coloured precipitate.

**2.10 Xanthoprotein**
One ml of the Gloriosa tuber the extracts were treated with HNO3. A few drops of liquid ammonia were also added. Reddish orange or reddish pink colour is formed. Its indicates that the presence of xanthoprotein.

**2.11 Phenols**
One or two ml of tuber extracts were treated with 2ml of water with four drops of FeCl3 reagent was added. The blue color was appeared and its showed that the presence of phenolic content.

**2.12 Saponins**
Saponins were detected by using the help of froth test. One gram of the Gloriosa tuber sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was allowed to stand for 30 minutes. Honeycomb froth indicated the presence of Saponins.

**2.13 Steroids and terpenoids**
Few ml of the Gloriosa tuber extracts was evaporated and the residues were dissolved in 0.5ml glacial acetic acid followed by the addition 0.5ml chloroform and few drops of concentration $H_2SO_4$. The colours shows that green, red and violet indicated that the presence of steroids triterpenoids respectively.

**2.14 Starch**
A few ml of the *Gloriosa* tuber extracts 10% (W/V) of sodium hydroxide solution was added and then heated. Reddish brown colour precipitatation formed presence of reducing sugar.

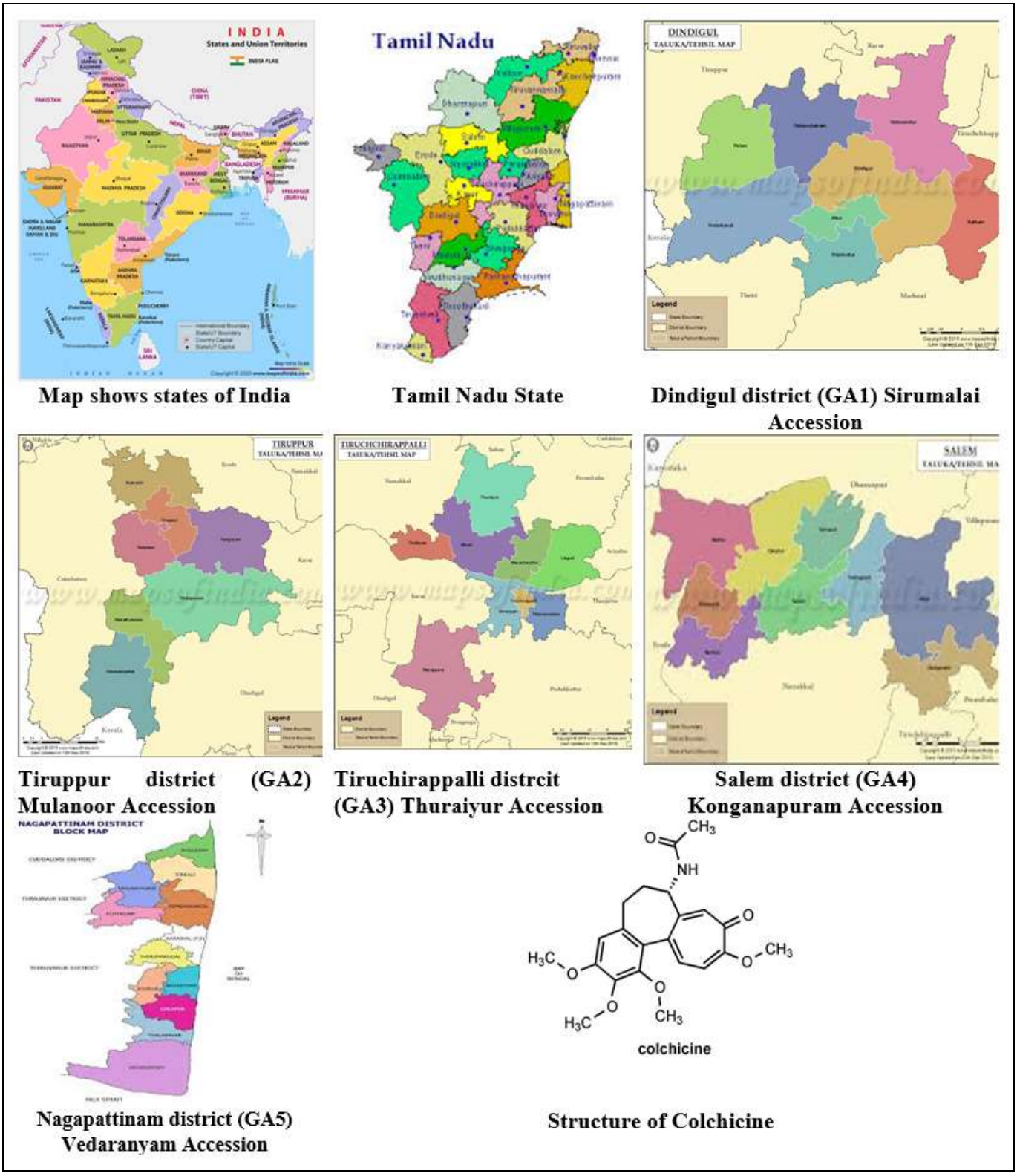


**Fig 1:** Map shows different districts of Tamil Nadu for *Gloriosa superba* L cultivated area selected for accession studies.

## 3. Results and Discussion

For the determination of Colchicine content in *Gloriosa superba* L tubers, five different accessions such as Sirumalai (GA1), Mulanoor (GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyam (GA5) belongs to the districts such as Dindigul, Tiruppur, Trichy, Salem and Nagapatinam respectively. These places are different accessions and climatic conditions. The methanolic extracts obtained from *Gloriosa superba* L, contain different kinds of quantitative analysis, phytochemicals such as alkaloid, flavonoids, glycosides, xanthoproteins, phenols, saponins, steroids, starch and terpenoids (Table. 2). There are nine active phyochemicals are reported form all five different accessions of *Gloriosa superba*. Quantitative phytochemical analysis of methanolic extracts of *Gloriosa superba* was reported.

**Table 1:** Different accessions of *Glorisoa superba* L cultivated places in Tamil Nadu State, India

| S. No. | Accession | Place | Location (Latitude and Logitude) |
|---|---|---|---|
| 1. | (GA1) | Sirumalai | 10º 11' 39.28" N and longitude is 77º 59' 48" E. |
| 2. | (GA2) | Mulanoor | 10.77º N and 77.72ºE. |
| 3. | (GA3) | Thuraiyur | 11º 8'29.2380" N and longitude is 78º35'40.100 |
| 4. | (GA4) | Konganapuram | 11.58ºN, 77.92ºE. |
| 5. | (GA5) | Vedaranyam | 10º22'77 27.15" N and longitude is 75º 51' 27.66" E. |

**Table 2:** Qualitative estimation of different accessions of *Gloriosa superba* L in Tamil Nadu State

| S. No | Phytocompound | Sirumalai (GA1) | Mulanoor (GA2) | Thuraiyur (GA3) | Konganapuram (GA4) | Vedaranyam (GA5) |
|---|---|---|---|---|---|---|
| 1. | Alkaloid | +++ | +++ | + | +++ | +++ |
| 2. | Flavonoids | + | ++ | ++ | + | + |
| 3. | Glycosides | ++ | + | ++ | + | +++ |
| 4. | Xanthoprotein | +++ | +++ | + | +++ | +++ |
| 5. | Phenols | ++ | +++ | ++ | ++ | +++ |
| 6. | Saponins | + | ++ | + | + | + |
| 7. | Steroids | ++ | ++ | + | + | ++ |
| 8. | Starch | +++ | ++ | +++ | +++ | +++ |
| 9. | Terpenoids | ++ | ++ | ++ | ++ | +++ |

**Table 3:** Quantification of Colchicine in *Gloriosa superba* in Tamilnadu State

| S. No. | Accession | Peak area of the HPLC chromatogram (a) | Concentration of standard injected in HPLC (µl) (b) | (a X b) = C | Peak area of standard in HPLC (d) | Quantity of colchicines (mg/g/dr.wt) a X b d |
|---|---|---|---|---|---|---|
| | Sirumalai (GA1) | 29400.390 | 0.02 | 588.0078 | 1145.097 | 0.51 |
| | Mulanoor (GA2) | 65243.823 | 0.02 | 1304.876 | 1145.097 | 1.1 |
| | Thuraiyur (GA3) | 42448.081 | 0.02 | 848.961 | 1145.097 | 0.741 |
| | Konganapuram (GA4) | 93919.665 | 0.02 | 1878.393 | 1145.097 | 1.64 |
| | Vedaranyam (GA5) | 71291.856 | 0.02 | 1425.837 | 1145.097 | 1.20 |

**Table 4:** Quantity of Colchicine in different accessions of *Gloriosa superba* L in Tamil Nadu State

| S. No. | Accession | Retention time (min) | Peak area (mvs) | Peak height (mv) | Quantity of colchicines (mg/g/dr.wt) |
|---|---|---|---|---|---|
| 1 | Sirumalai (GA1) | 3.232 | 29400.390 | 26.979 | 0.51 |
| 2 | Mulanoor (GA2) | 3.254 | 65243.823 | 34.678 | 1.1 |
| 3 | Thuraiyur (GA3) | 3.184 | 42448.081 | 19.077 | 0.741 |
| 4 | Konganapuram(GA4) | 3.275 | 93919.665 | 33.077 | 1.64 |
| 5 | Vedaranyam (GA5) | 3.265 | 71291.856 | 27.092 | 1.20 |

The quantity of Colchicine estimated with the help of HPLC Chromatogram peak. The quantification of Colchicine content in all five different accessions of *Gloriosa Superba* L in Tamilnadu state were analyzed with the help of following formula. The quantity of Colchicine were observed in all five different accessions of *Gloriosa Superba* L tubers (Table. 3)

$$Cp\ (S) = \frac{Ap\ (S)}{Ap\ (St)} \times Cp(st)$$

Cp (S) = The concentration of solute in the mixture

Ap (S) = The concentration of the peak of the sample in HPLC Chromatogram

Ap (St) = The area of peak of standard in HPLC Chromatogram

Cp (St) = The concentration of standard used for injecting HPLC

The chromatogram with standard are represented with five different accessions such as accessions such as Sirumalai (GA1), Mulanoor (GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyam (GA5) are provided. Based on peak level the amount of colchicines is calculated. The quantity of Colchicine was recorded in 0.51 mg/g/dr.wt in Sirumali (GA1) accessions, 1.1 mg/g/dr.wt was recorded in Mulanoor (GA2) accession, 0.741 mg/g/dr.wt was recorded in Thuraiyur (GA3) accession. In Konnganapuram (GA4) Accession 1.64 mg/g/dr.wt and Vedaranyam (GA5) 1.20 mg/g/dr.wt were recorded in *Gloriosa superba* L accessions. There values are high in GA4 and GA5 accessions.

The screened bioactive phytochemicals are known as primary compounds of antioxidants, which is protect against the damage caused by an oxidative stress induced by free radicals (Sudarmani, 2006) [33]. Plants have an ability to produce aromatic substances, which are phenols or their derivatives of oxygen (Geissman, 1963) [10]. Nearly 12,000 secondary metabolites are isolated from medicinal plants so far (Schultes, 1978) [26]. In the present investigation, methanolic extracts of all five different accessions of *G.superba* L. reveals that the maximum yields of phytochemicals. Meantime, *G.superba* yields good quality of results in phytochemicals (Devi and Femina, 2012) [7].

Phytochemical analysis of tubers or dried roots have showed that the presence of colchicines, glycoside, gloriosine, long chain fatty acids, flavonoids, tannins, alkaloids, 3-O-demethylcolchicine-3-O-αD-glucopyranoside, colchicocide, 1,2-didemethyl colchicine, luterlin, Glucoside, β and γ Lumicolichicines, β silosterol, Flucoside, 2,3- didemethyl colchicine, N-formyl deacetyl colchicines, tannins, superbine, 2-hydroxy-6-methoxy benzoic and salicylic acid (Shanmugam *et al*., 2009) [28].

Moreover, several studies have been reported that colchicine treatment induced cytomixis, nuclei movement from one plant cell to another via intercellular channels or intercellular bridges. Cytomixis in almond and peach reported by Singhal and Kumar (2008) [30], bioactive constituents identified through HPTLC method (Soodan and Wafai (1987) [31]; *In vitro* induction of tetraploids by colchicines (Souza *et al*., 2015) [32]. During microsporogenesis, cytomixis has been described to take place in a variety of flowering plants (Datta *et al*., 2005 [5]; Singhal and Kumar, 2008 [30]; Sidorchuk *et al*., 2007 [29]; Rai *et al*., 2010 [22]; Kumar and Srivastava, 2013) [13]. Ortiz *et al*. (2013) [18]. This is because the colchicine may have influenced the cytokinin activity, which is essential for plant development as described by Deikman and Ulrich (1995) [6].

## 4. Conclusion

Medicinal plants are an important source of biologically active compounds then natural products and generally used for various treatments. The five different accessions of *Gloriosa superba* L showed different kinds of phytochemicals present in the tubers. The methanolic extracts reveals that the different phytocomponents *Gloriosa superba* tuber. The main alkaloid Colchicine was recorded in Konganapuram (GA4) accession and followed by Vedaranyam (GA5) accession. The Colchicine is used as good medicine and contains medicinal properties to treat various diseases in human beings. Further in order to find out quantification of Colchicine content in *Gloriosa superba* L is useful for cultivation practices in specific accessions and recommended to farmers.

## 5. Acknowledgement

The authors are thankful to Indian Institute of Crop Processing Technology (IICPT), (Ministry of Food Processing Industries, Government of India, Tanjore) and Central Electrochemical Research Institute (CSIR), Karaikudi Tamil Nadu to carry out the work successfully.

## 6. References

1. Antoccia A, Tanzarella C, Modesti D, Degrassi F. Cytokinesis-block micronucleus assay with kinetochore detection in colchicine-treated human fibroblasts. Mutat. Res-Fund. Mol. M. 1993; 287(1):93-99.
2. Banerjee MK, Kalloo G. Sources and inheritance of resistance to leaf curl virus in *Lycopersicon*, Theor. Appl. Genet. 1987; 73:707-710.
3. Chitra R, Rajamani K. Genetic variability of Kazhappai kizhangu (*Gloriosa superba* L) in Tamilnadu assessed using morphological and biochemical fruits, Journal of Trophical Agriculture. 2009; 7(1 – 2):77-99.
4. Daniel GO, Azonin J, Bastida J, Viladomat F, Codina C. Seasonal and spatial variations of alkaloids in *Merendeva montana* in relation to chemical defense and phenology. J.Chem. Ecol. 2003; 29:1117-1126.
5. Datta AK, Mukherjee M, Iqbal M. Persistent cytomixis in *Ocimum basilicum* L. (Lamiaceae) and *Withania somnifera* (L.) Dun (Solanaceae). Cytologia. 2005; 70(3):30-313.
6. Deikman J, Ulrich M. A novel cytokinin-resistant mutant of *Arabidopsis* with abbreviated shoot development. Planta. 1995; 195:440-449.
7. Devi NN, Femina W. GC-MS analysis of *Gloriosa superba* medicinal plant of Tamilnadu. Journal of Pharmacy Research. 2012; 5(1):343-345.
8. Dvoracova S, Sedemera P, Potesilova H, Santavy F, Simanek V. Alkaloids of *Gloriosa superba* L. Collect. Czech. Chem. 1984; 49(6):1536-1542.
9. Gautam N, Kumar G. Consequences of colchicine induced inter meiocyte connections in *Helianthus annuus*. Caryologia. 2013; 66(1):65-69.
10. Geissman TA. Flavonoid compounds, tannins, lignins and related compounds. In M. Florkin and E. H. Stotz (ed.), Pyrrole pigments, isoprenoid compounds and phenolic plant constituents. Elsevier, New York, N.Y. 1963; (9):265-33.
11. Hutching A, Terblanche SE. Observations on the use of some known and suspected toxic lilliflorae in Zulu and Xhosa medicine. S.Afr. Med. J. 1989; 75:62-69
12. Kruckeberg AR. An essay: the stimulus of unusual geologies for plant speciation. Systematic Botany. 1986; 11:455-463.
13. Kumar G, Srivastava N. Induced cytomictic variations in pollen mother cells of *Sesbania cannabina* Poir. J Cent. Eur. Agric. 2013; 14(3):872-880.
14. Le Rocek LG, Robbertise PJ. Tuber ontogeny, morphology and vegetative reproduction of *Gloriosa superba* L. S Afr. J Botany. 1994; 60(6):321-324.
15. Molad Y. Update on colchicine and its mechanism of action. Curr. Rheumatol. Rep. 2002; 4 (3):252-256.
16. Narayanaswamy T, Thirunavukkarasu T, Shyamala P, David E. A review on some poisonous plant and their medicinal values, Journal of Acute Diseases, 2014, 85-89.
17. Nicola D, Thomas JL, Henry RWP. Mechanism of Action of Colchicine in the Treatment of Gout. Clinical Therapeutics. 2014; 36(10):1465-1479.
18. Ortiz JPA, Quarin CL, Pessino SC, Acuña C, Martínez EJ, Espinoza F, Pupilli F. Harnessing apomictic reproduction in grasses: what we have learned from Paspalum. Ann. Bot. 2013; 112(5):767-787.
19. Paul Jasmine JA, Balakrishnan V. Intra specific analysis of *Gloriosa superba* (L). through ISSR finger printing and DNA sequencing of ecotypes collected from different accessions of Tamil Nadu state, India. Res. Plant Biol. 2018; (8):21-26.
20. Paul Jasmine JA, Sundari T, Balakrishnan V. Analysis of Physicochemical characteristics of soil from five different accessions of *Gloriosa superba* L.cultivating area. The I J of Ana Exper Modal Ana. 2020a; 12(3):1982-1995.
21. Paul Jasmine JA, Sundari T, Balakrishnan V. Phytochemical analysis of *Gloriosa superba* L., using GC-MS from five different ecotypes of Tamil Nadu state, India. Curr Bot. 2020b; (11):1-6.
22. Rai PK, Kumar G, Tripathi A. Induced cytomictic diversity in Maize (*Zea Mays* L.) inbred. Cytol. Genet. 2010; 44(6):334-338.
23. Raja Naika H, Lingaraju K, ManjunathK, Danith K, NagarajuG, Suresh D, NagabhushanaH. Green synthesis of CuO nanoparticles using *Gloriosa superba* L, extract and their antibacterial activity. J. of Taibah Unversity for Science. 2015; 9:7-12.
24. Reddy RA, Balkwill K, Mclellan T. Are plant taxa found on the Witwatersrand serpentine ecotypes and

substrate – generatalists? South African Journal of Botany. 2012; 80:81-95.
25. Sarin YK, Jamwal PS, Gupta BK, Atal CK. Colchicine from the seeds of *Gloriosa superba* L. Cur – Sci. 1974; 43:87.
26. Schultes RE. The kingdom of plants. In WAR. Thomson (ed.), Medicines from the Earth. McGraw-Hill Book Co., New York, N.Y, 1978, 208.
27. Seza O, Isabelle KP, Ahmet G. Colchicine resistance and intolerance in familial mediterranean fever: Definition, causes, and alternative treatments, Seminars in Arthritis and Rheumatism. 2017; 47:115-120.
28. Shanmugam H, Rathinam R, Chinnathambi A, Venkatesan T. Antimicrobial and mutagenic properties of the root tubers of *Gloriosa superba* linn. (Kalihari). Pakistan Journal of Botany. 2009; 41(1):293-299.
29. Sidorchuk YV, Deineko EV, Shumny VK. Peculiarities of cytomixis in pollen mother cells of transgenic tobacco plants (*Nicotiana tabacum* L.) with mutant phenotype. Cell Tiss. Biol. 2007; 1(6):570-576.
30. Singhal VK, Kumar P. Impact of cytomixis on meiosis, pollen viability and pollen size in wild populations of Himalayan poppy (*Meconopsis aculeate* Royle). J Biosci. 2008; 33(3):371-380.
31. Soodan AS, Wafai BA. Spontaneous occurrence of cytomixis during microsporogenesis in almond (*Prunus amygdalus* Batsch) and peach (P. Persika Batsch). Cytologia. 1987; 52:361-364
32. Souza VF, Pagliarini MS, Valle CB, Bione NC, Menon MU, Mendes-Bonato AB. Meiotic behavior of *Brachiaria decumbens* hybrids. Genet. Mol. Res. 2015; 14:12855-12865.
33. Sudarmani G. Identification of bioactive constituents of *Albizia amara* using FT-IR, HPTLC and GC-MS. International Journal of Chemical and Pharmaceutical Analysis, 2016, 3:1.
34. Tambong JT, Sapra VT, Garton S. *In vitro* induction of tetraploids in colchicine-treated cocoyam plantlets. Euphytica. 1998; 104:191-197.