Original Research Article

Journal of Biological Research & Biotechnology

Bio-Research Vol. 16 No.1; pp.1002-1016 (2018). ISSN (print): 1596-7409; eISSN (online):2705-3822

Effects of crude ethanolic extract of *Gloriosa superba* L. tuber, on root growth and root tip mitosis, of *Allium sativum* L.

[§]Udengwu OS and Nwafor CM

Genetics and Plant Breeding Laboratory, Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka, 410001, Enugu State, Nigeria.

[§]Corresponding author: Dr Udengwu OS. Email: obi.udengwu@unn.edu.ng, Tel: +2348037723300, +2348032034068

Abstract

Colchicine is an alkaloid produced by some members of the Liliaceae family which include *Gloriosa superba* L. Apart from its other medicinal uses, it is used for both cytological and plant breeding studies. Absence of pure colchicine in Nigeria has retarded research progress in the above-mentioned areas of research. In this study, the genotoxic and cytotoxic effect of ethanolic crude extract of *G. superba* tubers, obtained by freeze-drying method, on *Allium sativum* meristematic roots were examined; to explore the possibility of using it, in research, in the absence of pure colchicine. The mitotic index of the roots treated with aqueous crude extract concentrations of 500mg/L, 1000mg/L and 2000mg/L for 3, 6, 12 and 24 hours, showed that there was significant reduction in the number of dividing cells with time. There were evidences of disturbed mitotic phases, C-metaphase, precocious chromosome movement and anaphase bridges, which increased as concentration and duration of treatment increased. Distilled water and 8-hydroxyquinoline were used as negative and positive controls. *A. sativum* roots exposed to the crude extract concentrations for 3, 6, 12, 24, 48 and 72 hours showed significant inhibition of root growth which increased with concentration and duration of treatment. The results obtained from this study indicate that the crude colchicine contained in *G. superba,* is an effective tubulin binder, which can arrest cells at the metaphase stage and is therefore recommended for cytological studies in Nigeria and other underdeveloped economies, in the absence of pure colchicine.

Key words: Root growth, root tip, mitosis, *Gloriosa superba, Allium sativum,* colchicine

https://dx.doi.org/10.4314/br.v16i1.2 Journal Homepage: ***http://www.bioresearch.com.ng.***
Publisher***: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.***

INTRODUCTION

Gloriosa superba Linn is an annual climbing herb with perennial tuberous root that is found naturally in Africa, India and South-eastern Asia. It is now found distributed widely throughout the tropics and even in the temperate countries (Dounias, 2006). The plant is known for its many medicinal uses which include use in the fight of rheumatism, gout, leprosy, arthritis, etc. (Jain and Suryavanshi; 2010). Its wrong use was noted by many scholars which could cause poisoning resulting to death (Fernando and

Widyaratna, 1989; Dounias, 2006; Maroyi and Maesen, 2011; George, 2012). The plant is called many names depending on the location. *Gloriosa superba* Linn is at times called Flame Lily, Fire Lily, Glory Lily, Superb Lily and Climbing Lily (Jain and Suryavanshi; 2010). In Hindi, it is called Karihari. In South Africa, it is called Mkalamu or Kimanja nouchawi (Dounais, 2006) and in some parts of Igboland, it is known as Akandimmuo because the tubers often resemble a human finger.

Gloriosa superba is used medicinally because it contains many metabolically active components which include carbohydrates, fixed oil and fat, phenolic compounds, tannin, flavonoids, alkaloids etc. (Pawar *et al.*, 2010). The main active compound that makes this plant very useful is an alkaloid called colchicine. This compound was first isolated by Zeisel in the year 1883 from *Colchicum autumnale* (Sharma and Sharma, 1972). Colchicine was found to disturb the cell cycle by inhibiting the cell mitotic division at metaphase. When this happens, it will result in polyploidy in the plant as well as cause the chromosome of the plant to shorten and thicken. This makes it easier for the morphological features of chromosomes to be studied (Evans, 2009). The treatment of meristematic cells with this compound, to make them competent for cytological study is termed pre-treatment. Pre-treatment is very important in the study of chromosome numbers as well as morphology (Udengwu and Akaneme, 2010). These chemicals called tubulin binders work at low concentrations. For colchicine, 0.002M is normally used. Other pre-treatment chemicals include .02M 8-hydroxyquinoline, 0.05-4% veratrine and 0.5-1% choral hydrate (Sharma and Sharma, 1972).

A major setback in using these chemicals in cytological and plant breeding work is that pure supplies are very expensive, and they are unavailable as they are not usually produced in the country. Incidentally, the plants that produce these chemicals are limited in the sub-Saharan Africa. They include *Colchicium autumnale* L., *Iphigenia* spp and *Gloriosa superba* (Dounias, 2006). According to Dounias (2006), an increase in demand for colchicine will lead to adulteration as only 3 plants are the tropical source of colchicine in Africa and the countries where they occur include Nigeria, Cote d'Ivoire, Burkina Faso, Burundi, Zimbabwe, Cameroon, Tanzania, Kenya, Zambia, Senegal, Ethiopia, Somalia and South Africa. Also, the cost of importation of high-quality grade could be quite expensive.

Consequently, it becomes imperative that local sources of these pre-treatment chemicals should be explored. The most readily available plant that has been established to contain colchicine is *Gloriosa superba* Linn. The tuber of the plant has to be dug up from the ground by the beginning of the month of June. This is very important as the level of colchicine is said to be in high concentration, about 0.25%, at this period (Fernando and Widyaratna, 1989). The presence of colchicine and other antimitotic compounds present in the plant have a lot of uses in both plant and animal research. The binding of these chemicals at the tubulin inhibit the chromosomes segregation during meiosis in eukaryotic cells. When this occurs, half of the resting gametes will contain double the usual chromosome number while half will contain no chromosome. This will lead to embryos with double the usual number of chromosomes. While this is fatal in animals, it frequently results in plants that are larger, hardier, having more desirable features and fast growing.

Taylor *et al*. (2004) stated that colchicine has the ability to render infertile hybrids fertile, an example is seen in the case of breeding triticale from wheat (*Triticum* spp) and rye (*Secale cereale*). Wheat is usually tetraploid and rye diploid; their triploid would be infertile. However, colchicine induces fertile hexaploid *Triticale*. Colchicine-induced polyploidy is known to cause seedless fruit as seen in plantain, banana and watermelon (Wilson and Hunt, 1994). Evans (2009) reported that colchicine can induce polyploidy in root tips of plant by exposing meristematic apices to colchicine solution. Seeds can also be soaked in colchicine solution before planting to induce polyploidy. Since Basak *et al*., (2012) had shown that the colchicine content of *G. superba* tubers from different agroclimatic zones of India differed, it is important that our indigenous *G. superba* species should be explored to characterize their colchicine content, which might have some advantages over the ones found in other parts of the world where the plant grows.

This current study was initiated to develop a protocol for obtaining crude metabolically active antimitotic substances from the tubers of *G. superba* and evaluate their effectiveness as antimitotic agents, which can be used in place of the hard to come-by and very expensive pure

colchicine. This is a prelude to local extraction and characterization of pure colchicine found in this plant to boost diverse research efforts that require pure colchicine as a major chemical compound.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Gloriosa superba plants, which are climbers were easily identified at the following four locations at the Nsukka campus of University of Nigeria- the Botanic garden, Zoological garden, Ezeopi and Odenigwe the previous year based on their characteristic multi-coloured flowers with star shaped corolla (Fig 1).

Since the tubers are perennial organs while the shoots are annual in nature, the tubers (Fig. 2) were dug up from the four locations during the month of June, 2016, when the plants were still at the early stage of growth during the rainy season. After the harvesting, 2.4 kg of the bulked tubers were washed and freeze-dried for two days in a deep freezer, located at the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Teaching hospital, at a temperature of -10^{o} C. This followed the protocol of Bharahthi, *et al*. (2006).

Crude Extraction Procedure

From the bulked freeze-dried tubers, 800 g was weighed and ground with pestle and mortar. The macerated particles were mixed with 300 ml of absolute ethanol for 12 hours. The mixture was made into a paste using a Kenwood blender. This was filtered using a cheese cloth and the filtrate was mixed with 150 ml ethanol and filtered again. The filtrate was mixed with 150 ml distilled water and filtered. The filtrate was re-filtered five more times to remove all particles. After that the residue was allowed to decant and the supernatant was gently poured away. A milky-white powder was seen at the bottom of the 500 ml Pyrex conical flask. The powder was heated in a Gallenkamp electric oven at a temperature of 60^{o} C, for 12 hours to ensure that all traces of ethanol and water were removed. The crude extract powder was stored in a Gallenkamp refrigerator at 4^{o} C for future use.

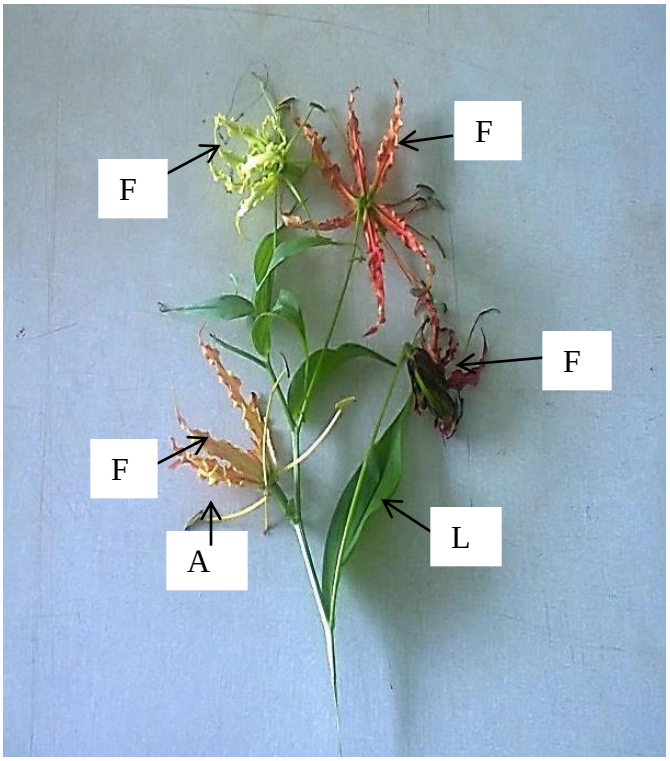

Fig. 1: Twig of *Gloriosa superba* showing multi-colored flowers (FL), Fruit set (FS), Leaves (LV) and Anthers (AN) (Mag. X 1/3)

Figure 2: Human Foot-shaped *Gloriosa superba* tuber (Mag. x 1/3)

Growing of *A. sativum* bulbs for antimitotic and cytotoxicity studies

Twenty-four medium sized fresh and healthy *A. sativum* bulbs used for the studies were bought from Shoprite Super Market in Enugu, South East Nigeria. The *A. sativum* bulbs were placed directly on top of the small-sized 80 ml plastic tumblers filled with distilled water such that the discoid stems of the *A. sativum* bulbs were in contact with water. The plastic tumblers were wrapped with black nylon sheets to prevent the roots from getting exposed to light (Fig. 3). The *A. sativum* bulbs were left for 3 days to allow them to start rooting (Fiskesjo,1985). After 6 days, when the roots were 5 cm long on the average (Fig 4), they were removed from distilled water and placed into the different treatment solutions and the controls. Table 1 gives the details of different solutions used for the studies. Solution A, which is only distilled water, is the negative control. Solution B which is .02 M 8 Hydroxyquinoline is the positive control, while solutions C, D and E represent the three concentrations of the crude extract formed by dissolving differently 500mg, 1000 mg and 2000 mg of the extract in 1000 ml of distilled water. They were labelled T1- half strength, T2- full strength and T3- double strength, respectively. There were three replications per treatment, and they were randomized in a CRD, giving a total of 15 experimental units (Figure 3).

Growing of *A. sativum* bulbs for root growth studies

Twelve healthy *Allium sativum* bulbs purchased form Shoprite Enugu, were placed on top of 80 ml small sized plastic tumblers filled with distilled water. They were placed in a dark cupboard in our Genetics and Biotechnology laboratory. The water was changed every day. After six days of planting, the bulbs were found to have produced numerous roots (Fig. 4) as well as green juvenile leaves. They were then separated into cloves before being placed in Pyrex test tubes wrapped with sheets of black polythene to shield them from light (Fig. 5). The test tubes contained the five different treatment solutions and the controls whose compositions were detailed in Table 1 and were explained above under (Growing of *A. sativum* bulbs for antimitotic and cytotoxicity studies)**.** There were six replications per treatment and the tubes were randomized in a CRD, giving a total of 30 experimental plants plus six additional plants maintained in distilled water which were set aside for possible replacement of non-surviving cloves after their separation.

Pre-treatment of root tips of the *A. sativum* for antimitotic and cytotoxicity studies

The pre-treatment protocol followed the method described by Udengwu and Akaneme (2010). The various bulbs of the *Allium sativum* which were maintained in 80 ml plastic tumblers containing distilled water were introduced into T1, T2, T3, C1 and C2 treatment solutions as set up in the experimental design in Fig. 3 to fully expose the roots to the treatment solutions. They were kept on the laboratory bench in our Genetics and Biotechnology laboratory. Four root-tips, approximately 1 cm long were harvested from the three replicates of each of the treatments after 3, 6, 12 and 24 hours. All the harvested roots per treatment were mixed up in labelled specimen bottles and washed with distilled water two times before fixation.

Fixation: The washed root-tips after pre-treatment were fixed in Carnoy's solution, 1:3 acetic acid and ethanol. The fixed root-tips in the specimen tubes were stored for 12 h in a Gallenkamp standing refrigerator at a temperature of 4^{o}C. After fixation, the root tips were stored in 70% ethanol and kept on specimen tube racks on the laboratory bench ready for hydrolysis.

Hydrolysis: The root tips were hydrolysed after washing three times with distilled water,

Table 1: Formulation of the solutions used for the studies

Name of solution	Weight of Crude *Gloriosa* extract	Volume of distilled water used	Concentration produced	Role played by solution
Solution A	Nil	1000 ml	Nil	Negative Control
Solution B	Nil	Nil	.02 M 8-Hydroxyquinoline	Positive Control
Solution C	500 mg	1000 ml	Half strength	Treatment (T1)
Solution D	1000 mg	1000 ml	Full strength	Treatment (T2)
Solution E	2000 mg	1000 ml	Double strength	Treatment (T3)

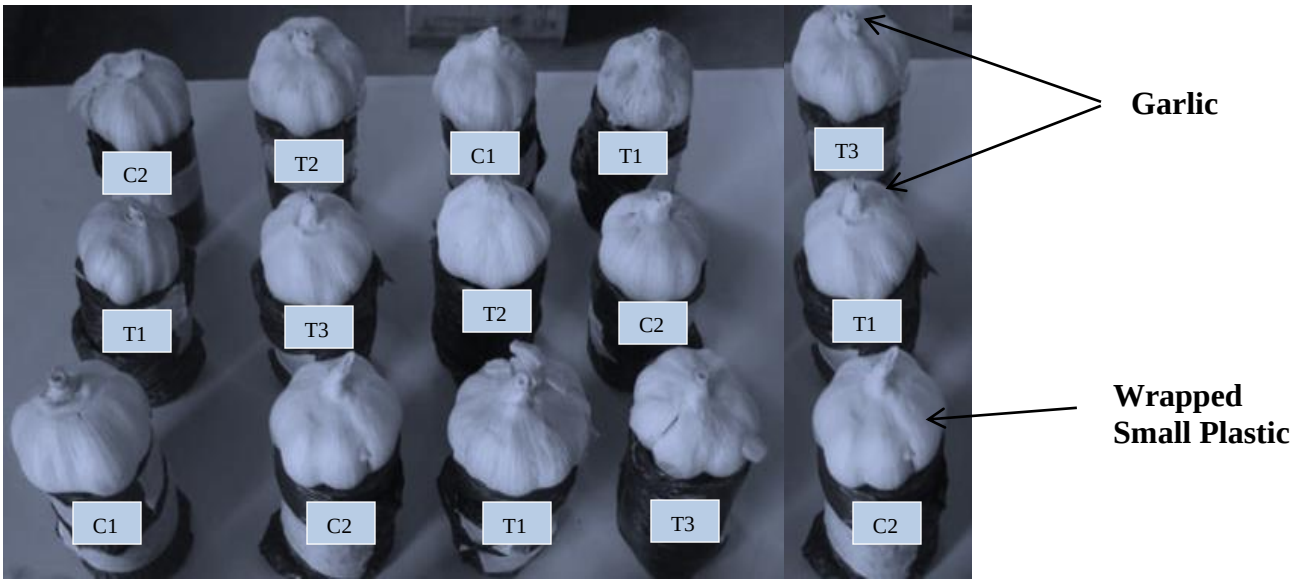

Figure 3: CRD design for bulbs for antimitotic and cytotoxicity studies (Mag x 1/3)

according to the method described by Sharma and Sharma (1972). Eight ml of 0.1N HCL was added to a specimen-tube immersed in a Gallenkamp Water bath set at 60^{o}C. The root tips were dropped inside the specimen tubes and left in the Water bath for 8 minutes. At the end of hydrolysis, the diluted acid was poured out and the root tips washed three times with distilled water to wash off the acid. The root tips were then emptied into labelled petri dishes according to the treatments, ready for use for squashing.

Slide preparation and study:
Two root tips were randomly picked from each of the treatments and placed on clean transparent glass slides placed on the laboratory bench. The milky tips of the two roots per slide were cut off while the other parts of the roots were discarded. Two drops of aceto-carmine were placed on top of the two root tips per slide before squashing

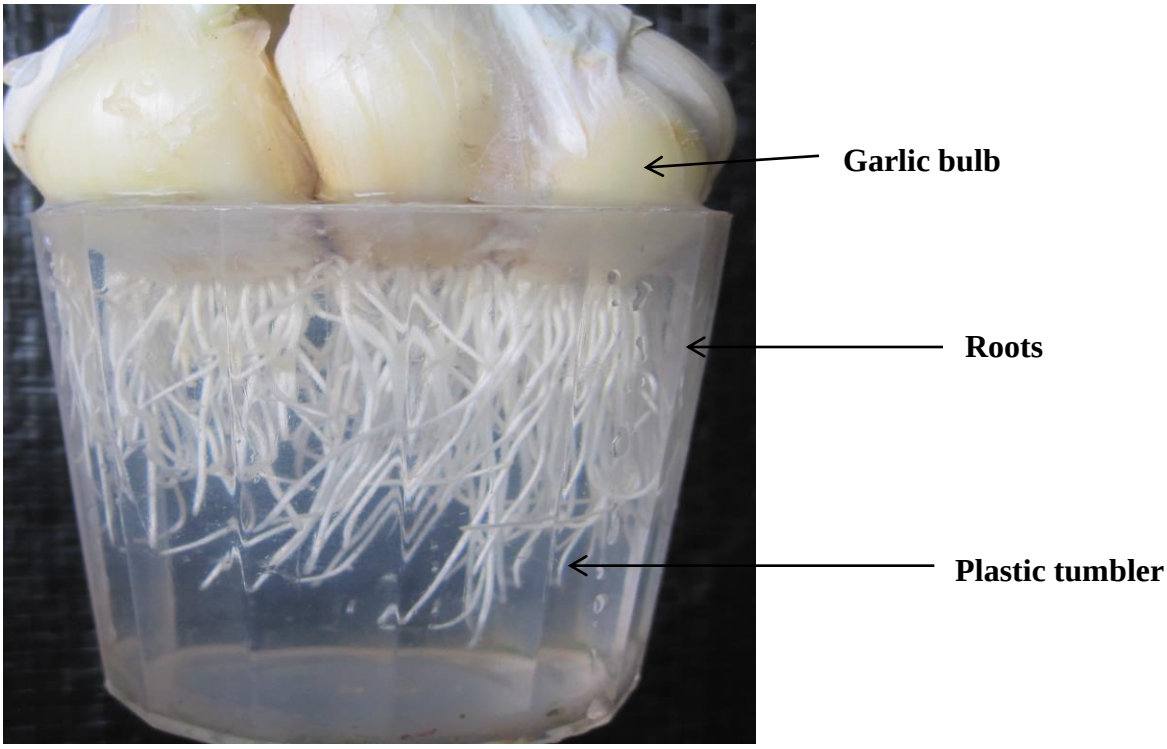

Figure 4. Profuse production of roots by *A. sativum* bulb for antimitotic and cytotoxicity studies

Figure 5. Experimental set up of cloves of *A. sativum* for root growth studies

was carried out with the aim of a prep-needle. Squashing continued till all the lumps became fine particles. The squashed preparations were left pick up the stain for 4 minutes. The preparations were then covered by gently lowering a cover slip with the aid of a forceps.

This was done carefully to avoid trapping of air bubbles. The preparations were allowed to stay for 5 minutes before the excess stain was blotted out. The blotting was done by sandwiching the slide between a folded filter paper and the right thumb was used to press the cover slip. Care was taken not to allow the cover slip to slide on the slide. Microscopic examination was made at x 40 magnification and promising slide preparations were sealed with nail varnish for antimitotic and cytotoxicity studies. All necessary data were collected, and photomicrograph of important cells were taken.

Data collection and statistical analysis

After studying the slides and obtaining the raw data, the following formulae were used for calculating important parameter: -

A. Mitotic Index (MI) = $\frac{\text{Number of dividing cells}}{\text{Total number of cells observed}} \times 100$

B. Percentage number of abnormal cells= $\frac{\text{Number of abnormal phase cell}}{\text{Total number of dividing cells}} \times 100$

C. Percentage abnormal phase = $\frac{\text{Number of abnormal cell phase}}{\text{Total number of cells in that phase}} \times 100$

Analysis of variance (ANOVA) was used to analyse the data obtained for the dividing cells. Genstat software was used for the statistical analysis.

Measurement of growing roots

The growth of the roots was measured at 0, 3, 6, 12, 24, 48 and 72 h intervals with a flexible plastic measuring tape. The rooting cloves were lifted from each solution and the lengths of the three longest roots were taken. Formation of any abnormalities was noted.

RESULTS AND DISCUSSION

The cytological protocols employed were found to be quite effective for they made the cells competent for easy observation of both the mitoclassic and chromatoclassic effects of the crude extract on the meristematic cell of *A. sativum*. Fixation prepared the surface of chromosomes to absorb stains as well as render tissues soft for squashing (Udengwu and Akaneme; 2010). Hydrolysis resulted to the clearing of the cytoplasm of substances that could pick up stains and obscure the chromosome when viewing. With proper hydrolysis, only the chromosome was able to pick the stain thereby making them easier to study (Sharma and Sharma, 1972).

The ANOVA for dividing cells (Table 2) shows that while the concentration (C) effect of the crude extracts and Control 2(CON 2) were very highly significant (P≤.05), the effect of duration of treatment (T) was highly significant, with the interactive effect between concentration and time (C x T) being significant. This is an indication that the crude extract of *G. superba* contained metabolically active antimitotic compounds that affected the mitotic processes of *A. sativum*. Other studies (Jason *et al.,* 2014, Indah *et al*.,2018, Ramya Sree and Thoppil, 2018) have confirmed exhibition of anti-mitotic properties by aqueous extracts of *Gloriosa superba* and other plants containing natural colchicine analogs. In separating the means of the main effect of concentration (Table 3) for total number of dividing cells (TNDS); only the mean for control 2 (CON 2) differed significantly from the three plant extract concentrations:- 500mg/L (C1) , 1000mg/L (C2), 2000mg/L (C3) and control 1 (CON1).

Table 2: Analysis of variance of dividing cells

Source of variance	d. f	S.S	M.S	V.R	F. Pr
Concentration	4	90161.0	22540	19.84***	0.001
Time	3	18779.0	6260	5.31**	0.003
C×T	12	29998.0	2500	2.20*	0.031
Residual	40	45439.0	1136		
Total	59	184378			

Mitotic index (MI), decreased from CON1 to T3, for concentration though the decrease did not differ significantly between CON1 and C1 as well as between C2 and C3. They however differed significantly with CON 2. Celik and Aslanturk (2010) reported that mitotic index below 22% will have lethal impact on the *A. sativum.* They explained that mitotic index measures the proportion of cells at the mitotic phase and its inhibition could be interpreted as cell death or delay in cell proliferation-kinematics. The observed that decrease in mitotic index is an indication that the crude ethanolic extracts of the *G.superba* are mitodepressive and genotoxic to *A. sativum* roots.

There was no significant difference between C1, C2, C3 and CON1, CON2 for number of dividing cells at prophase (NDCP). For the effects of concentration on number of dividing cells at metaphase (NDCM), there was significant difference between CON2 and C1, C2, C3 and CON1. However, CON1 and C1 did not differ significantly just like C2 and C3 did not also differ significantly. The effect of concentration on number of dividing cells at anaphase (NDCA) indicated that there were no significant differences between C1, C2 and C3, but they all differed significantly with CON1 and CON 2, which equally differed from each other. For number of dividing cells at telophase (NDCT), CON1 differed significantly with all others, while C1, C2 and C3 did not differ significantly from each other, but they differed significantly with CON 2. Lastly, for number of abnormal dividing cells (NADC), CON 1 differed significantly from all others, while C1, C2 and CON 2 did not differ significantly from each other but differed significantly with C3.

Separation of the means of main effect of time on TNDC (Table 4) showed that there were no significant differences over the time durations. This was also the case for NDCP and NADC. For (MI) only 6 and 12 h did not differ significantly. For NDCM all differed significantly except for 6 and 24 h. Whereas 3 and 6 h did not differ significantly for NDCA and NDCT, they differed significantly for 12 and 24 h. The above results indicate that the mito-depressive effects of the crude extracts significantly increased more with increase in concentration than with increase in duration of treatment. This is equally supported by the cytotoxicity studies.

Figure 6 (a-d) shows typical cells at prophase, metaphase, anaphase and telophase stages of mitotic division. These serve as standards for identifying observable aberrations at different stages of mitotic division. Fig. 6 e shows a C-metaphase (arrowed) caused by treatment with T2 for 2 h. Fig. 6 f shows disturbed prophase (orange arrow) caused by T2 after 6 h treatment. The black arrow of the same Fig 6 shows C-anaphase equally induced by the 6 h treatment of *A. sativum* roots with T2. Mild C- metaphase (Fig. 6 g and h) were induced by treatment with T1 for 24 h. On the other hand, T3 used to treat roots for 12 h resulted to C-metaphase (Fig. 6 i) while treatment with same T3 for 24 h resulted to the inducement of C-metaphase (Fig. 6 j). Control 2 induced C-metaphase (Fig. 6 k) and very sticky telophase (Fig. 6 l) after it was applied to the roots for 24 h. Sticky metaphase (Fig. 6 m) was induced by T2 after 24 h of treatment. C-metaphase (black arrow Fig. 6 n) and sticky metaphase (orange arrow) were both induced after treatment with C2 for 12 h. The presence of these abnormal mitotic stages is an indication of the presence of C-mitotic substances (mainly colchicine) in the crude extracts, whose effects largely increased with increase in concentration and also with duration of treatment. This agrees with earlier studies (Jason *et al.,* 2014, Indah *et al*., 2018, Ramya Sree and Thoppil, 2018). Furthermore, the results showed that the milder forms of aberrations like disturbed phases occurred at the lower concentrations after shorter durations of treatment, while the more severe aberrations like C-mitosis, sticky metaphase and anaphase occurred at higher concentrations over longer durations of

substances (Kazuo *et al.,* 1983) depended upon the concentration of the substances as well as the duration of treatment. Interestingly (Figs.6f and 6h) showed that there were chromosomal disturbances even at premetaphase stages, showing that contrary to many reports (Izumi *et al.*, 1983; Ekong *et al*., 2014; Ramya Sree and Thoppil, 2018), that colchicine and colchicine analog substances merely attack the spindle apparatus at metaphase, thereby preventing cells from transiting from metaphase to anaphase; their effects actually affect cells at earlier stages of the cell cycle. This is in line with the reports of Fitzgerald and Brehaut, 1970; Mueller *et al.,* 1971, who cautioned that the effects of colchicine on the cells are not always limited to the metaphase spindle.

Additionally, Münzbergová**,** (2017) reported on the prolonged aftereffects of colchicine application in polyploidization studies and opined that the strong positive effect of colchicine application on plant performance could be due to the colchicine application exerting strong selection pressure on the plants, which only the fittest plants survive. The report further stated that it is possible that colchicine application affects the epigenome of the plants and hence the adverse effects of colchicine, and possibly of other substances used to create synthetic polyploids, must be considered up to the second generation of plants, in studies using synthetic polyploids.

From Table 5, which presents data for the interactive effects of C x T on M1, NDCP, NDCM, NDCA, NDCT and NADC, it can be seen that the interactions had their effect on MI. The interactive effects on NDCP were significant between the single digit hours and the double-digit hours at CON 1, C1, C2. The above results show that NDCP had the highest number of diving cells. This could be because cells at the prophase stage were not disturbed as much as cells at other stages of cell division. For metaphase, the NDCM were second to the NDCP but thereafter NDCA and NDCT recorded a dramatic decrease in the number of diving cells. This is an indication that due to the antimitotic effects of the C-mitotic crude extracts and CON 2, cells were accumulated at metaphase with many not advancing to the anaphase and telophase stages. This is due to the disorganization of the spindle apparatus in many of the cells at metaphase thereby preventing cell division from advancing to stages beyond metaphase. This agrees with the findings of Sarathum *et al*, 2010; Koyani and Saiyad 2011; Tiwari and Mishra, 2012; Ekong *et al.,* 2014. When the mean effect of CON 2, the positive control, was compared with the three treatments, it could be seen that the mean number of cells at the various mitotic stages for CON 2 was higher than that of any of the treatments. This could be because the crude extracts contain other metabolically active compound that amplified their anti-mitotic effects coupled with the fact that the actions of colchicine and 8-hydroxyquinoline, though a well-known c-mitotic compounds differ. These views are supported by the reports that *Gloriosa superba* apart from containing colchicine also contains another toxic alkaloid, Gloriosine (Angunawela and Fernando, 1971; Sarin *et al*., 1974) as well as other compounds like lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine and N-formyldeacetylcolchicine (Chulabhorn *et al*., 1998). Burley (1964), working on the karyotype of the sitka spruce, *Picea sitchensis*, observed that at five hours of pretreatment, the total haploid complement of the chromosome had a 41.6% contraction due to the effect of 1% aqueous solution of colchicine but at twenty-four hours in 0.002M 8- hydroxyquinoline, the total haploid compliment showed only a 37.2% contraction. Further research work is needed to use crude extracts of *Gloriosa superba* with lower concentrations to compare their effects with the effect of the ideal positive control, 1% pure colchicine solution, when available, alongside .002M 8-hydroxquinoline, which is cheaper and more readily available. This will give an idea of the concentration of the crude extract whose antimitotic effect will be closest to that of pure colchicine, thereby filling the vacuum created by non-availability of pure colchicine as well as the use of fake colchicine which when employed in research tantamount to pure waste of time and resources. This is however not to lose sight of the fact that the ultimate aim will be to develop or adapt economical and sustainable protocols that will result to local production of pure colchicine. The works of Pandey and Banik, 2012; Jason *et al.,* 2014 and Agrawal and Laddha, 2019, have shown that this option is achievable, and this will be explored in future research.

Table 3: Means of main effect of concentration on total number of dividing cells (TNDS), mitotic index (MI), no. of dividing cells at prophase (NDCP), no. of dividing cells at metaphase (NDCM), no. of dividing cells at anaphase (NDCA), no. of dividing cells at telophase (NDCT) and no. of abnormal dividing cells (NADC)

Concentration	Means of main effect of concentration on:						
	TNDS	MI	NDCP	NDCM	NDCA	NDCT	NADC
Control 1	227.8[a]	7.348[b]	125.7[a]	64.2[b]	22.58[b]	17.50[b]	15.67[a]
500mg/L	218.5[a]	7.287[b]	124.2a	61.2[b]	16.33[a]	12.58[a]	23.17[b]
1000mg/L	218.7[a]	6.912[a]	98.6[a]	52.3[a]	14.33[a]	10.75[a]	25.42[b]
2000mg/L	206.8[a]	6.806[a]	98.6[a]	50.3[a]	11.67[a]	8.17[a]	32.17[c]
Control 2	313.4[b]	10.707[c]	150.2[a]	83.1[c]	45.00[c]	26.67[c]	22.75[b]
$LSD_{0.05}$	27.81	0.2313	61.14	7.25	5.145	3.549	5.346

Means bearing different letters along each column differ significantly using $LSD_{0.05}$.

Table 4: Means of main effect of time on total number of dividing cells (TNDS), mitotic index (MI), no. of dividing cells at prophase (NDCP), no. of dividing cells at metaphase (NDCM), no. of dividing cells at anaphase (NDCA), no. of dividing cells at telophase (NDCT) and no. of abnormal dividing cells (NADC)

Time (hr)	Means of main effect of time on:						
	TNDS	MI	NDCP	NDCM	NDCA	NDCT	NADC
3	264.4[b]	8.888[c]	131.9a	72.7[c]	28.40[b]	17.40[b]	25.87[a]
6	240.0[a]	7.955[b]	126.1a	64.5[b]	25.60[b]	18.20[b]	24.93[a]
12	226.0[a]	7.475[b]	110.7a	57.5[a]	16.07[a]	14.00[a]	22.23[a]
24	217.7[a]	6.929[a]	109.2a	54.2[a]	17.87[a]	10.93[a]	22.20[a]
$LSD_{0.05}$	24.87	0.2069	54.68	6.49	4.602	3.174	4.782

Means bearing different letters along each column differ significantly using $LSD_{0.0}$

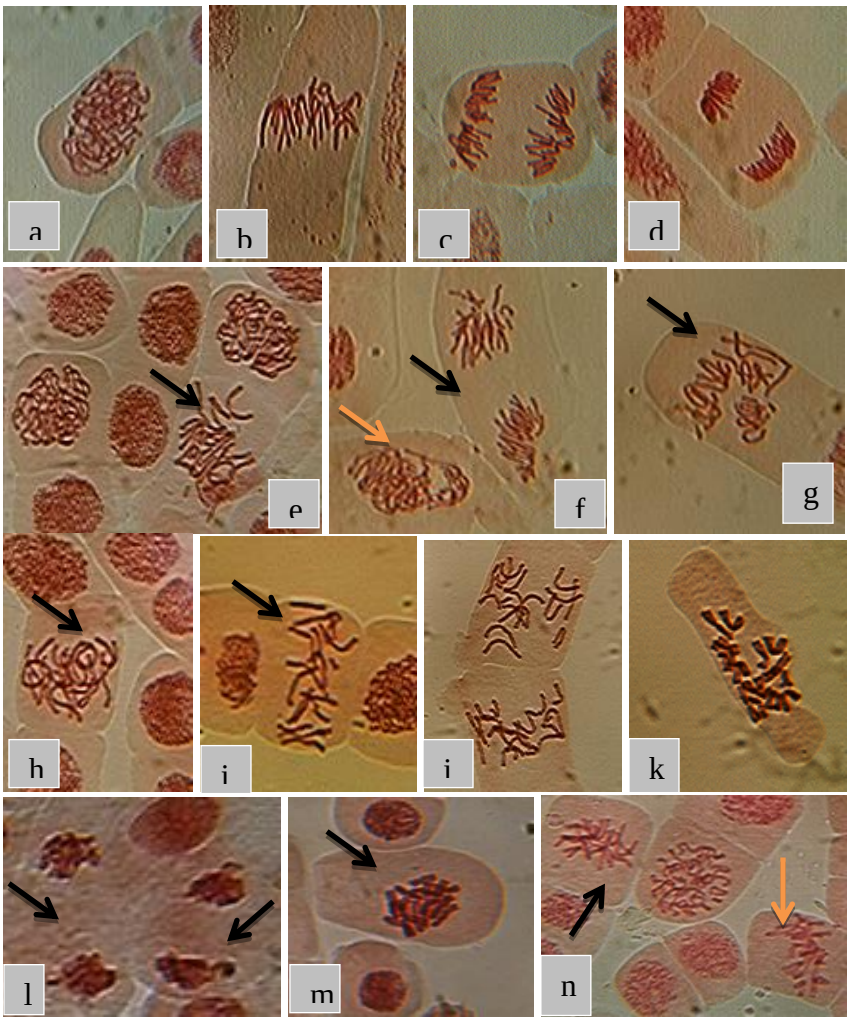

Fig. 6 (a). Normal Prophase, (b). Normal Metaphase
(c). Normal Anaphase, Fig. 6 d. Normal Telophase
(e). C-Metaphase induced by 1000mg/L after 12 h treatment
(f). Disturbed prophase (Orange arrow) induced by 1000mg/L after 6 h treatment
C-Anaphase (Black arrow) induced by 1000mg/L after 6 h treatment
(g). Mild C-Metaphase induced by 500mg/L after 24h treatment
(h). C-Prophase induced by 500mg/L after 24 h treatment
(i). C-Metaphase induced by 2000mg/L after 12 h treatment
(j). Two C-Metaphase stages induced by 2000mg/L after 24 h treatment
(k). C-Metaphase induced by Control 2 after 24 hours
(l). Very sticky telophase caused by Control 2 after 24 hours
(m). Sticky Metaphase caused by 1000mg/L after 24 hours of treatment
(n). C-Metaphase (Black arrow) and Sticky metaphase (Orange arrow)
induced by Control 2 after 12 hours of treatment.

Table 5: Summary of interactive effect of concentration and time on mitotic index (MI), dividing cells at prophase (NDCP) metaphase (NDCM), anaphase (NDCA), telophase (NDCT) and abnormal dividing cells (NADC).

Conc./Time	M. I.	Prophase	Metaphase	Anaphase	Telophase	ADC
Control 1						
3hrs	9.000^{g}	118.700^{a}	70.700^{b}	32.670^{c}	22.333^{b}	26.333^{b}
6hrs	7.390^{d}	75.000^{a}	63.700^{b}	26.333^{b}	32.333^{c}	30.333^{b}
12hrs	5.977^{b}	72.300^{a}	69.700^{b}	14.670^{a}	6.670^{a}	16.000^{a}
24hrs	4.657^{a}	237.000^{b}	40.700^{a}	16.670^{a}	8.670^{a}	20.000^{a}
500mg/L						
3hrs	8.443^{f}	136.300 ^{b}	56.300^{b}	20.000^{b}	15.333^{b}	25.333^{b}
6hrs	7.220^{d}	99.700 ^{a}	44.700^{a}	20.670^{b}	18.670^{b}	25.670^{b}
12hrs	6.173^{b}	87.000^{a}	62.700^{b}	9.333^{a}	4.333^{a}	21.670^{a}
24hrs	5.810^{b}	71.300^{a}	45.700^{a}	15.33^{a}	12.000^{b}	29.000^{b}
1000mg/L						
3hrs	7.930^{e}	143.700^{b}	64.000^{b}	11.000^{a}	6.000^{a}	12.000^{a}
6hrs	7.333^{d}	141.700^{b}	39.000^{a}	10.670^{a}	9.000^{a}	18.670^{a}
12hrs	7.210^{d}	111.000^{a}	50.700^{a}	15.333^{a}	9.333^{a}	30.000^{b}
24hrs	6.670^{c}	100.700^{a}	47.700^{a}	9.670^{a}	8.333^{a}	30.333^{b}
2000mg/L						
3hrs	7.143^{d}	88.300^{a}	65.300^{b}	18.670^{a}	12.000^{b}	30.333^{b}
6hrs	7.113^{c}	91.000^{a}	58.300^{b}	19.000^{a}	11.333^{a}	33.670^{b}
12hrs	8.453^{f}	121.000^{a}	71.000^{c}	12.333^{a}	14.333^{b}	32.333^{b}
24hrs	6.230^{b}	124.000^{b}	57.000^{b}	11.821^{a}	16.333^{b}	33.261^{b}
Control 2						
3hrs	11.923^{j}	143.300^{b}	107.00^{d}	59.670^{d}	31.333^{c}	17.000^{a}
6hrs	10.520^{i}	146.300^{b}	82.000^{c}	51.333^{d}	19.670^{b}	16.333^{a}
12hrs	9.563^{h}	154.700^{b}	68.700^{b}	28.670^{b}	20.000^{b}	11.670^{a}
24hrs	10.820^{i}	156.300^{b}	74.700^{b}	40.333^{c}	35.670c	17.670^{a}
$LSD_{0.05}$	0.463	122.270	14.500	10.290	7.098	10.692

Means bearing different letters differ significantly using $LSD_{0.05}$

The ANOVA for root length (Table 6) showed that a very highly significant difference occurred for concentration as well as for the interaction, C x T. Also, there was a highly significant difference for duration of treatment. Separation of the means for the main effect of concentration on root length (Table 7) showed that, it was only T1 and T2 that did not differ significantly. For the means of main effect of time on root length (Table 8) 48 and 72 h durations did not differ significantly from each other but differed significantly with all the other durations of treatment. From the interactive effects of concentration and time (C x T) on root length (Table 9), it was observed that concentration was the major contributor to the observed interactive effect for all the time durations The contributions of time were significant at 24, 48 and 72 h durations of treatment for C2. These results go to confirm the earlier ones obtained from the cytotoxic studies. This is as expected because the observed reduction in root growth which increased more with increase in concentration than with increase in duration of treatment is the aggregation of all the observations made at the cellular level, for it is the individual cells that make up the roots. This serves as a further confirmation that the leaf extracts contained biologically active compounds that interfered with the normal processes of cell proliferation that result to root growth. This is in agreement with the findings of Narain and Raina (1975); Gul *et al*. (2006), Konuk *et al.* (2007) and El-Nashar and Ammar (2016) but differs from that of Vijayalakshmi and Singh (2011) who stated that, colchicine increased the vegetative parameters like root length, shoot length

.

Table 8: Means of main effect of time on root length

Time	Mean
0hr	1.377[b]
3hrs	1.288[b]
6hrs	1.201[a]
12hrs	1.151[a]
24hrs	1.095[a]
48hrs	1.071[a]
72hrs	1.045[a]
$LSD_{0.05}$	0.1837

Means bearing different letters differ significantly using $LSD_{0.05}$

Table 9: Interactive effect of concentration and time on root length

	Time						
Conc.	0	3hrs	6hrs	12hrs	24hrs	48hrs	72hrs
Control1	1.273[b]	1.288[b]	1.315[b]	1.333[b]	1.355[b]	1.365[b]	1.327[b]
500mg/l	1.252[b]	1.282[b]	1.233[b]	1.278[b]	1.353[b]	1.315[b]	1.287[b]
1000mg/l	0.943[a]	0.945[a]	0.745[a]	0.940[a]	0.970[a]	0.947[a]	0.975[a]
2000mg/l	0.687[a]	0.687[a]	0.940[a]	0.795[a]	0.810[a]	0.792[a]	0.792[a]
Control 2	1.068[a]	1.152[b]	1.243[b]	1.243[b]	1.518[b]	2.022[c]	2.505[d]
$LSD_{0.05}$ = 0.4107							

Means bearing different letters differ significantly using $LSD_{0.05}$.

epicotyl length, hypocotyl length, petiole length, intermodal length and number of lateral roots of Cluster bean *Cyamopsis tetragonoloba*. It therefore appears that the effect of colchicine on root growth varies with different plant species. Further studies involving different types of plants are needed to draw conclusions on this.

There was no change in colour of the root tip with increase in time for roots grown in T1. However, there was gradual increase in the colour of the root tips of *A. sativum,* from very light yellow to light yellow, with increase in concentration, T1, T2, T3 and C2. The reasons for this is not clear but it might not be unconnected with the accumulation of non-functional or almost dead cells which could not continue with the normal process of mitotic division, for the growth of the roots, due to the severe interference of the antimitotic *Gloriosa* crude extracts. Similar findings were made by Konuk *et al.* (2007) who reported that the root tip of *Allium cepa* darkened, became thick and gel-like after exposure to boron at a concentration as low as 100ppm.

Conclusion

The present study has shown that the presence of colchicine and other antimitotic compounds make *Gloriosa superba* crude extract an effective pre-treatment chemical for cytological studies in the absence of the pure colchicine. The mitodepressive and the chromatoclassic effect was seen by the diverse chromosomal abberations and inhibition of growth of the roots of *Allium sativum* bulbs. However, the cytological effect of the alkaloid present in the plant will be more effective if the alkaloid is obtained in a pure form. This is the next level of researchwork in this area. With the local production of pure colchicine, indigenous plant breeding efforts, through polyploidization, will receive a giant boost. Currently this plant is utilized locally in the ethnobotanical healthcare system, especially in the rural areas, mostly among the poor. As a result of the observed serious cytological effects on the chromosomes of *A. sativum,* caution must be exercised when using the plant as medicine, as it has the potentials to cause severe cell poisoning to the

consumer, where the life saving dosage is exceeded Further cytological and biochemical studies using animal model could further highlight the potential dangers abuse of this plant could pose.

References

Agrawal, P., and Laddha, K. (2019). Extraction of colchicine from *Gloriosa superba* tubers: a comparison of conventional and microwave-assisted extraction, *Journal of Microwave Power and Electromagnetic Energy,* doi: 10.1080/08327823.2019.1570450.

Angunawela, R.M. and Fernando, H.A. (1971). Acute ascending polyneuropathy and dermatitis following poisoning by tubers of *G. superba*. Ceylon Medical Journal, 16: 233-235.

Basak, U.C., Dash, D. and Mahapatra A.K. (2012). Estimation of Colchicine in tubers of *Gloriosa superba* L. originated from different agroclimatic zones of Odisha, India. International *Journal of Pharmacognosy and Phytochemical Research* 4(3): 157-161.

Bharathi, P., Philomina, D. and Chakkaravarthi, S. (2006). Antimitotic effect of colchicine from six different species of *Gloriosa superba* in Onion roots (*Allium cepa*). *Journal of Medical Science*, 6(3):420-425.

Burley, J. (1964). Karyotype of Sitka Spruce, *Picea sitchensis* (Bong). *Carr. Silvae Genet*.ics, 14: 127-137.

Celik, T. A. and Aslanturk, O. S. (2010). Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extract with *Allium t*est. *Journal of Biomedicine and Biotechnology*, 18(9): 252-258.

Chulabhorn, M., Somsak, R., Hunsa, P., Somcliai, P., Surang, E., Phannipha, C., Tasanee, T., Stitaya, S., and Pichas, P. (1998). Biodiversity and natural product drug discovery. *Pure and Applied Chem.*, 70: 2065-2072.

Dounias, E. (2006). *Gloriosa superba* L. Schmelzer, G. H. and Gurit-Farkim, A. (Editors). PROTA (Plant Resource of Tropical Africa/ Resource Vegetales de L'Afrique Tropicale). http://database.prota.org/search.htm Accessed 2 August 2018.

Ekong, N. J., Akpan G. A. and Udo I. J. (2014). Comparative effects of colchicine, 8-hydroxyquinoline and paradichlorobenzene on arm ratio of mitotic chromosomes of *Allium cepa* L. *International Journal of Medicinal Plants and Alternative Medicine* 2(2): 21-26.

El-Nashar, Y.I. and Ammar, M.H. (2016). Mutagenic influences of colchicine on phenological and molecular diversity of *Calendula officinalis* L*. Genetics and Molecular Research* 15 (2):1-15 gmr.15027745.

Evans,W.(2009).*Trease and Evans Pharmacognosy 16th Edition* Benjamin Cumming International Publishers, San Francisco, 869pp.

Fernando, R. and Widyaratna, D. (1989). *Gloriosa superba L*. Leon, C. (Editor). *International Programme on Chemical Safety Poisons Information Monograph* 245.

Fiskejo, G. (1985). The *Allium* Test as a Standard in Environmental Monitoring. *Hereditas*, 102:99-112.

Fitzgerald, P. H., and Brehaut, L. H. (1970). Depression of DNA synthesis and mitotic index by colchicine in cultured human lymphocytes. *Experimental Cell Research*. 59 :27.

George, M. D. (2012). Death related to herbal therapy for joint pains-a rare case of *Gloriosa superba* poisoning. *Journal of Clinical and Diagnostic Research,* 5(2): 379-380.

Gul, T., Kaymak, F. and Muranli, F. D. P. (2006). Genotoxic effects of Avenoxan on *Allium cepa* L. and *Allium sativum* L. *Caryologia*, 59(3): 241-247.

Indah, R., Susilowati, A., Widyastuti, Y. and Yunus, A. (2018). Artemisinin content of *Artemisia annua* L. treated by *Gloriosa superba* seeds' water extract. *Asian Journal of Pharmaceutical and Clinical Research,* 11(8): 147-149

Izumi, K., Kanazawa, H., and Saho T. (1983). Colchicine-like effect of propyzamide on the root meristematic cells of *Vicia faba* L. Journ. *Fac. Sci., Hokkaido Univ. Ser. V (Botany),* 13 (1): 17-24.

Jain, A. P. and Suryavanshi, S. (2010). *Gloriosa superba* Linn. -A Pharmacological review. *International Journal of Pharmaceutical. Research and Development*, 2(3): 24-26.

Jason, R. J., Mohamed, I. M., Selva, G. R. and Sankar, M. (2014). Quantification of colchicine in various parts of *Gloriosa superba* by HPLC. *Journal of Chemical and Pharmaceutical Sciences Special Issue* 2: 54-55. Available @ www.jchps.com.

Konuk, M., Liman, R. and Cigerci, I. H.(2007). Determination of genotoxic effect of boron on *Allium cepa* root meristematic cells. *Pakistan Journal of Botany*, 39(1):73-79.

Koyani, P. R. and S.S. Saiyad (2011). Study of effect of colchicine exposure on length of chromosome during mitosis. *Journal of the Anatomical Society of India* 60(2) 177-180.

Maroyi, A. and van der Maesen, L.G.D. (2011). *Gloriosa superba* L. (Colchicaceae): Remedy or poison. *Journal of Medicinal Plant Research*, 5(26):6112-6121.

Mergen, F. and Burley, J. (1963). *Abies* spp Karyotype Analysis. *Silvae Genetica*, 13: 63-68.

Mueller, G. A., Gaulden, M. E. and Drane, W. (1971) The effects of varying concentrations of colchicine on the progression of grasshopper neuroblasts into metaphase. *The Journal of Cell Biology*. 48: 253-265.

Münzbergová, Z. (2017). Colchicine application significantly affects plant performance in the second generation of synthetic polyploids and its effects vary between populations *Annals of Botany*:120 (2): 329-339,https://doi.org/10.1093/aob/mcx070**.**

Pandey, D. K. and Banik, R. M. (2012). Optimization of extraction conditions for colchicine from *Gloriosa superba* tubers using response surface methodology. *Journal of Agricultural Technology*, *8*(4):1301-1315.

Pawar, B. M., Wavhal, V. P., Pawar, N. D., Agarwal, M. R., Shinde, P. B. and Kamble, H. V. (2010). Anthelmintic Activity of *Gloriosa superba* Linn. (Lilaceae*). International Journal of Pharm. Tech. Research*, 2(2):1483-1487.

Ramya Sree, P. R. and Thoppil, J. E. (2018). C-mitotic potential of aqueous leaf extract of *Memecylon randerianum* SM & MR Almeida: A promising natural colchicine analog. *International Research Journal of Pharmacology* 9(11):115-118 http://dx.doi.org/10.7897/2230-8407.0911269.

Sarathum, S., Hegele, M., Tantiviwat, S. and Nanakorn M. (2010). Effect of concentration and duration of colchicine treatment on polyploidy induction in *Dendrobium scabrilingue* L. *European Journal of Horticultural Sciences.,* 75 (3). S. 123–127.

Sarin, Y.K., Jamwal, P.S., Gupta, P.K. and Atal, C.K. (1974). Colchicine from the seeds of *Gloriosa superba*. Current Sciences., 43: 87-87.

Sharma, A. K. and Sharma, A. (1972). Chromosome technique-theory and practice. butterworth and co publishers limited, Baltimore.575 pp.

Taylor, D. J., Green, N. P. O. and Stout, G. W. (2004). *Biological Science*. Soper, R. (Editor). Cambridge University Press, London. 984pp.

Tiwari, A. K. and Mishra, S. K. (2012). Effect of colchicine on mitotic polyploidization and morphological characteristics of *Phlox drummondi*. *African Journal of Biotechnology* 11 (39): 9336-9342.

Vijayalakshmi, A. and Singh, A. (2011). Effect of colchicine on cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]. *Asian Journal of Environmental Sciences,* 6(2): 171-174.

Wilson, J.H. and Hunt, T. (1994). *Molecular Biology of the Cell: The Problem Book.* Albert, B. (Editor). Garland Publisher, New York. 460pp.

Udengwu, O. and Akaneme, F. (2010). *Introductory Practical Genetics For Life Sciences*. *Faculty of Biological Sciences Publication and Colloquium Committee*. 53pp