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I.J. Udo
Department of Science and Technology, Akwa Ibom State Polytechnic, Nigeria

G.A. Akpan
Department of Botany and Ecological Studies University of Uyo, Nigeria.

N.J. Ekong
Department of Biological Science, Federal University, Wukari, Taraba State, Nigeria

Correspondence:
I.J. Udo
Department of Science and Technology, Akwa Ibom State Polytechnic, Nigeria

Cytotoxic effects of alcoholic extracts of 5 medicinal plants on mitosis in *Allium cepa* root tips after 12 h recovery from 24 h treatments

I.J. Udo, G.A. Akpan, N.J. Ekong

Abstract
The study was conducted to investigate the effects that 24 h treatments with extracts from 5 medicinal plants may have in *Allium* cepa root tips after 12 h recovery in distilled water, *Allium* cepa root tips were immersed in alcoholic at the concentrations of 0, 25, 50, 75 and 100 mg/ml, respectively, of the following plants: *Gnetum africanum*, Welw., *Lasianthera africana* P. Beauv, *Ocimum gratissimum* Linn., *Telfairia occidentalis* Hook F. and *Vernonia amygdalina* Del., used in herbal medicine. Results obtained show that the various concentrations of the extracts had toxic effects on the cells, which caused general significant reduction ($p<0.05$) in the mitotic index when compared with the control. Other effects were the accumulation of prophase and telophase stages. This may be due to lack of spindle fibres formation that would have introduced the cells to another stage, due to the interaction of the tested extracts. The use of the leafy extracts of these plants should be with caution.

Keywords: Medicinal plants, Alcoholic extracts, *Allium cepa*, mitosis, cytotoxic effect, 12h recovery.

1. Introduction
Medicinal plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs. The use of medicinal plants for treatment and management of diseases has been gaining prominence worldwide, especially in the developing countries where the rural population still depends on traditional healing methods (Apostolides *et al*., 1996). Scientific interest in medicinal plants has burgeoned in recent times due to the fact that synthetic drugs pose rising concerns about side effect and also of the incidence of antibiotic resistance especially among the antibiotic family in modern medicine (Lewis, 1977).
Natural plants are known to contain substances that can be used therapeutically and for the synthesis of novel drugs. Many of these phytochemicals seem to fight diseases and lower the rate of which they occur. (Harborne, 1990). Some of these plants used in herbal medicine practice are *Gnetum africanum* Welw., *Lasianthera africana* P. Beauv., *Ocimum gratissimum* Linn., *Telfairia occidentalis.* Hook F., *Vernonia amygdalina* Del. This paper reports an attempt to establish the cytotoxicity to these medicinal plants using the *Allium* cepa test.

2. Materials and Methods
Leaves of *Gnetum africanum, Lasianthera africana, Telfairia occidentalis, Ocimum gratissimum,* and *Vernonia amygdalina* were obtained from the Postgraduate Research farm in university of Uyo. The plants were identified at the Herbarium Unit of the Department of Botany and Ecological Studies, University of Uyo, where the voucher specimens have been deposited. The leaves were dried and pulverized, using mortar and pestle in the laboratory following the method of Mukhtar and Tukur (1999). Thereafter, alcoholic extracts of the plants were prepared using the method of Fatope *et al;* (1993). The extracts were serially diluted to the concentrations of O,25, 50, 75 and 100 mg/ml respectively.

2.1 Treatment of Onion root tips
The root tips of *Allium cepa* bulbs were immersed in the different concentrations of the plant extracts for 24 hour. Thereafter, the onion bulbs were returned to distill water for 12 hour to recover.

The roots were thereafter fixed in acetic acid and alcohol (1:3 v/v). After 24 h, it was hydrolyzed for 6 mins at 60 ^{0}C in NHCL. The root tips were cut, macerated and squashed in a drop of aceto-orcein on a clean slide. The slides were examined using high power objectives of the microscope (40x objectives). Two hundred cells were examined on each slide and three slides were prepared for each concentration. Mitotic index (M) as well as cells with abnormalities were recorded. The statistical evaluation was performed using one-way analysis of variance and where this was significant, the Least Significant Difference (LSD) analysis was used to separate the means.

3. Results

The extracts from the various test plants had toxic effects on cell division resulting in the reduction of the mitotic index.

24 h Treatments

(a) Mitotic Index

Table 1 shows the mean mitotic indices of the control and the various alcoholic extract concentrations of the medicinal plants after 24 hours treatment.

Analysis of variance of the mitotic indices indicated significant differences among the various concentrations of the extracts of G. *africanum*, *L. africana*, *O. gratissimum*, *T. Occidentalis and V. amygdalina.* Least Significant Difference (LSD) analysis showed that the mitotic index in root tips treated with the various concentrations of the alcoholic extracts of the medicinal plants was significantly reduced ($p < 0.5$) when compared with the control. However, there was no apparent trend in the reduction of mitotic index, but the data showed that this was most severe at the concentration of 75 mg/ml in the root tip treated with *O. gratissimum* (Table 1).

Table 1: Mean mitotic indices in the control and the various alcoholic extract concentrations of the traditional medicinal plants studied after 24 hours treatment

Mitotic index					
Concentration (mg^{-ml})	***G. africanum***	***L africana***	***O gratissimum***	***T occidentalis***	***V amygdalina***
0	18.36	18.36	18.36	18.36	18.36
25	9.34*	11.76*	12.88ns	12.08*	12.35*
50	10.46*	12.58ns	10.67*	10.57*	10.71*
75	12.00*	11.61*	8.80*	12.11*	12.99*
100	9.98*	11.36*	12.04ns	11.54*	13.16*
LSD(P< 0.05)	4.71	6.52	8.66	3.96	4.84

* = Significantly different from control Ns = Not signification different

(b) Cells in the Mitotic Stage

There was a noticeable decrease in the mean number of prophase cells in *O. gratissimum* treatments as the concentration of the alcoholic extract increased (Fig 1A).

Fig. 1B shows that the mean number of cells in the metaphase stage was strongly reduced to zero in *V. amygdalina* treatments at concentrations of 25, 75 and 100 mg/ml while in *T. occidentalis* treatments it was also reduced to zero at the concentration, except at the concentration of 50mg/ml. in the other words, *V. amygdalina* extracts had the most severe depressive effect at 25, 75 and 100 mg/ml treatments.

Fig ID shows that the mean number of cells in telophase was much reduced in *G. africanum* treatments at the concentration of 25 mg/ml and 75 mg/ml, and was noticeably much more reduced at the concentrations of 50 mg/ml and 100 mg/ml, respectively.

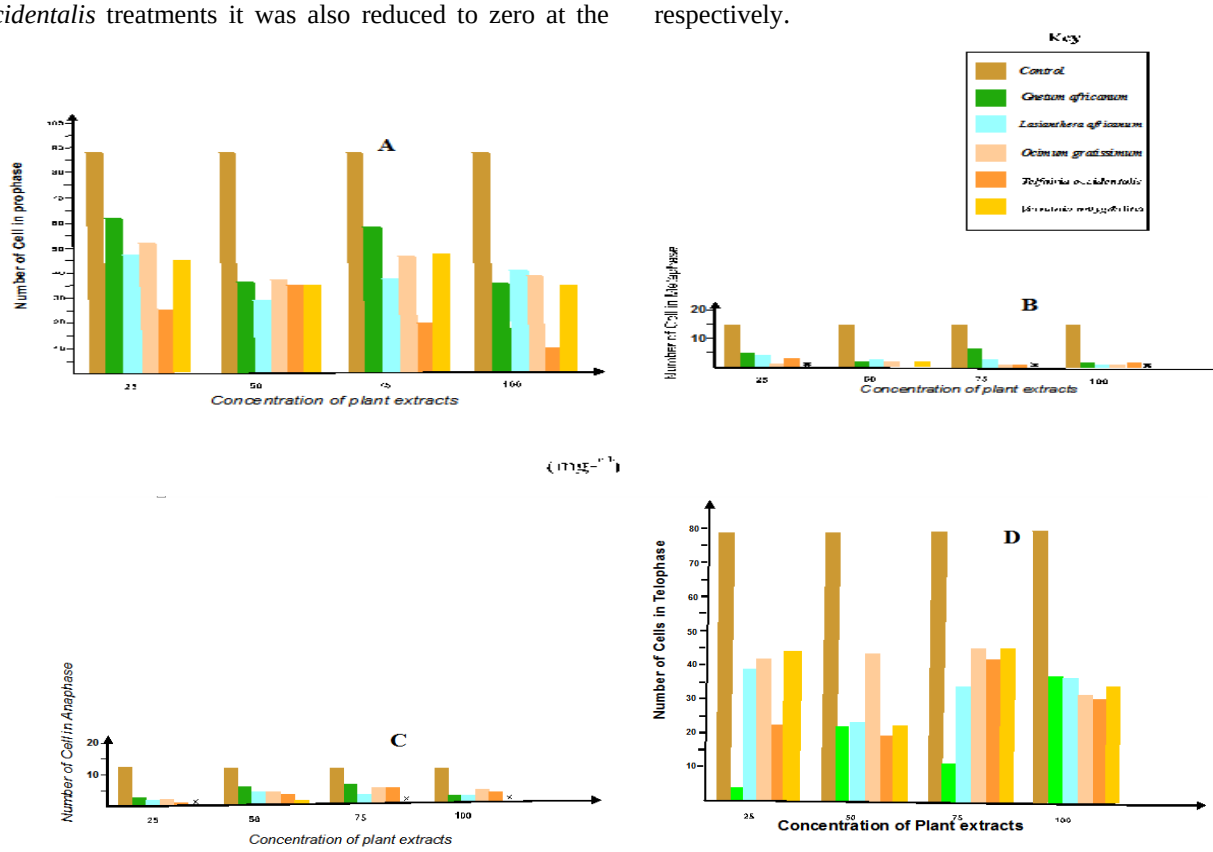

Fig 1: Bar charts of the mean number of cells at the different stages of mitosis in the various concentrations of the plant extracts after 24 hr treatment of *A. cepa* root tips: A. Prophase; B. Metaphase; C. Anaphase; D. Telophase

12 h Recovery

(a) Mitotic Index

Table 2 shows the mean mitotic indices in the control and in the various concentrations of medicinal plants after 12 hours recovery in distilled water.

Analyses of variance of the mitotic indices indicated significant differences among the various extract concentrations. The analyses indicated that in *G. africanum*, *L. africana* and *O. gratissimum* the mitotic index was significantly reduced ($p < 0.5$) at all concentrations when compared with the control. There was no apparent trend in the recovery treatment of 25mg/ml *G. africanum* and 75mg/ml in *L. africana.*

Table 2: Mean mitotic indices with the control and in the various concentrations of the alcoholic extracts of the traditional medicinal plant after 12 hours recovery in distilled water.

Concentration (mg^{-ml}) *occidentalis*	*G. africanum*	*L africana*	*O*	*T gratissimum*	*V amygdalina*
0	18.36	18.36	18.36	18.36	18.36
25	9.34*	10.98*	12.59*	13.54*	14.56ns
50	10.55*	11.56*	11.08*	12.24*	11.07*
75	11.88*	9.12*	12.04*	16.33ns	14.06*
100	11.14*	10.38*	11.30*	15.85ns	11.82*
LSD($P < 0.05$)	4.53	2.27	2.53	4.12	4.04

* = Significantly different from control Ns = Not signification different

(b) Cells in the mitotic stages

Fig. 2A shows that the mean number of cells in prophase was more strongly reduced with *L. africana* and *O. gratissimum* than with *G. africanum* and *V. amygdalina* after 12 hours recovery period. Treatments with *V. amygdalina* and *T. occidentalis* at 75 and 100 mg/ml concentrations showed a relatively higher number of prophase cells than treatments with the other medicinal plants.

Fig 2B shows the mean number of cells in the metaphase stage was highly suppressed with *G. africanum*, L. *africana*, *O. gratissimum*, *T. occidentalis* and *V. amygdalina treatments.*

Figure 2C shows that the mean number of cells in anaphase was strongly reduced with *O. gratissimum* at the 100 mg/ml than *L. africana*, *G. africanum* and *T. occidentalis* treatments.

Fig 2D shows that the mean number of cells in anaphase was not so strongly reduced with *V. amygdalina* as with the other treatments at the 75 and the 100 mg/ml treatments with 12 h recovery.

The prophase stage was found to have the highest number of cells in treatment with alcoholic extracts of the medicinal plants when compared with the control. This was closely showed by the telophase. From the data obtained it is evident that mitosis remained depressed after 12 h recovery from the treatments in distilled water.

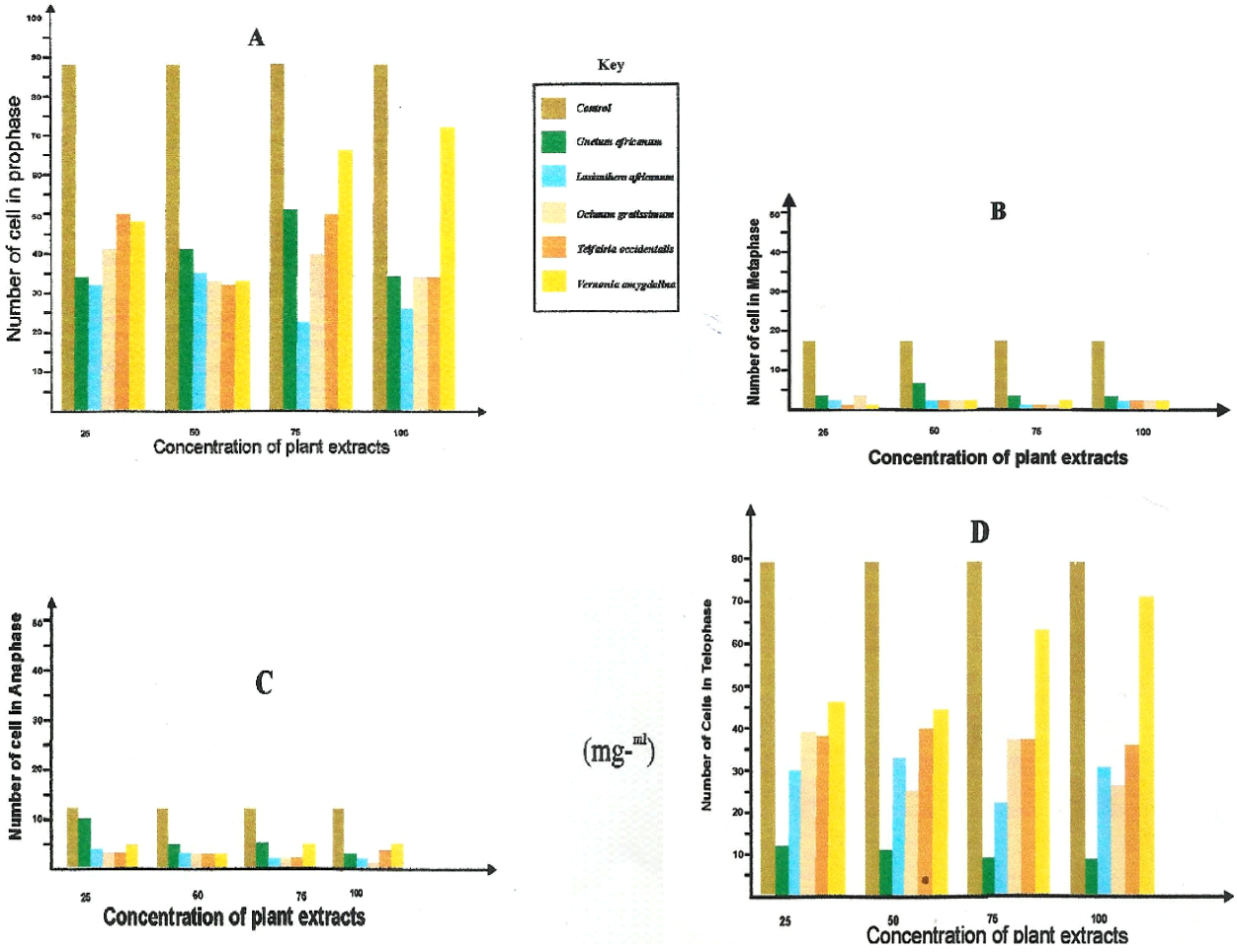

Fig 2: Bar charts of the mean number of cells at the different stages of mitosis in the various concentrations of the plant extracts after 24 hr treatment and 12 hr recovery in distilled water of *A. cepa* root tips: A. Prophase; B. Metaphase; C. Anaphase; D. Telophase

Fig. 3 shows the only abnormality that was observed in the root tips cells of *A. cepa* at all the concentrations of treatments with the alcoholic extracts of the various medicinal plants. This was the appearance of nuclear lesions characterized of clear areas in the nucleus.

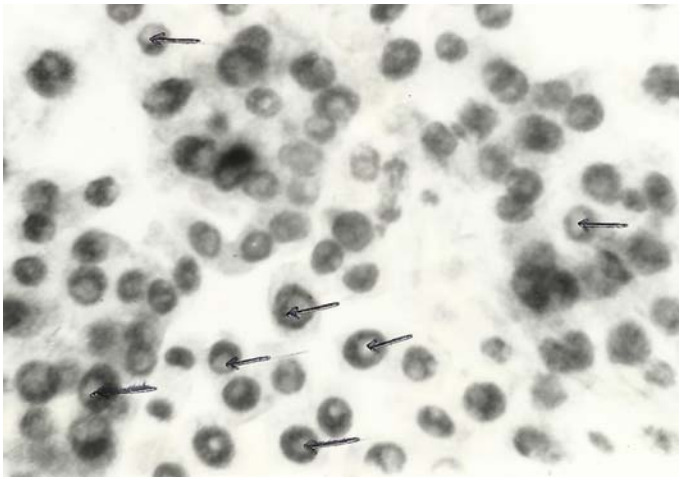

Fig 3: Nuclear lesions (arrowed) in interphase cells of onion root tips treated with concentration of 75 mg-^{ml} of the extract of *G. africanum*.

4. Discussion

The cytotoxic effects observed in this study were similar to the results of Itoyama *et al.,* (1997) and Grisolia *et al.,* (1995) which showed that a high concentration of any chemical will have an effect on the cell cycle, as has been shown for caffeine in *Drosophila prosaltan,* and *Pogostemon heyneanus* extracts *in A. cepa root tip* cells.

The effects of the extracts was most noticeable in the transition from the telophase to the prophase stage. This suggests that the effect of the extract was at the interphase stage when the cells were making preparations for the initiation of mitotic division. The stopping of the progression of cell division, though not total, was probably due to the inhibitory action of the extracts. Similar observations were made by Kabarity and Malallah (1980), Formina *et al.* (1989) and Udo *et al.,* (2014) with the root tips of *A cepa*. In this study, this reduction in mitotic progression may have been further enhanced by the plant extracts reducing the number of cells at metaphase and anaphase stages. The nuclear lesion observed may have been caused by the extracts affecting the protein components of the chromatin, leading to their dissolution, hence the lesions (Udo *et al*., 2014).

4.1 Conclusion

The alcoholic extracts of the five medicinal plants have been shown to have cytotoxic effects on. *A cepa*. root tip cells. They decreased the mitotic index and induced nuclear lesion at all concentrations. A 12h recovery in distilled water did not change the cytotoxic outcomes. In view of this, the use of these plants for herbal medicine practice should be with caution.

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