



Studies on mito depressive effect of leaf extracts of some medicinal plants on *Allium cepa* root tip assay

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Abstract

The mito depressive effect induced by these plant extracts of *Annona muricata* L. and *Morinda citrifolia* L. suggests that it has some effect on cell division of *Allium cepa* L. which may be due to the condition induced by the chemical components of the extracts. These plants can be used in the treatment of uncontrolled cell divisions, The present investigation also proves their potentialities to control cell division in an appropriate concentrations.

1. Introduction

The use of medicinal plants has always been part of human culture from time immemorial. Plants are known to contain substances that can be used medicinally and for the synthesis of novel drugs. Many of these phytochemicals seem to fight disease and lower the rate of its occurrence. A plant is said to be medicinal when one or more parts of it is used in the therapeutic action or it can be used as the precursor in the synthesis of useful drugs (Emmanuel, 2015). Use of such medicinal plant in the treatment of various ailments in developed as well as developing countries is deep rooted from ancient periods. These itself shows the widely acknowledged importance of medicinal plants with immuno-potential in the treatment of non-curable disease such as cancer (Abubaker *et al.*, 2007).

The world health organization estimates that up to 80% of the world's population relies on traditional medicinal system for some aspects of primary health care. Scientific interest in medicinal plants has emerged in recent times due to the fact that synthetic drugs pose noticeable side effects and also antibiotic resistance gained by causative organisms in modern medicine. Despite the profound therapeutic advantage possessed by some of the medicinal plants, some constituents of it have found to be potentially toxic, mutagenic, carcinogenic and teratogenic. However, the potential toxicity of herbs has not been recognized by the general public or professional groups of traditional medicine (Soetan & Aiyelaagbe 2009) and the importance of plant based studies for varying purpose are increasing day by day especially in the

field of medicine. In this study we have used the leaf extract of *Morinda Citrifolia* L to study the mitodepressive effect on *Allium cepa* root tip. The use of plant tissues, primarily root tip for studying the induction of chromosomal aberration is one of the oldest, simplest, most reliable and inexpensive method (Auti *et al.*, 2010). In the field of cytogenotoxic and mutagenicity studies, *Allium cepa* L. is the common plant species used for long years (Kuras *et al.*, 2006). Among plant cells *Allium* shows most similarity to the mammalian cells (El shabbaby *et al.*, 2003). A wide array of ethno-medicinal activities of *A. muricata* contributed to different parts of indigenous communities in Africa and South America extensively use this plant in their folk medicine. Traditional medicinal uses of *A. muricata* have been identified in tropical regions to treat diverse ailments such as fever, pain, respiratory and skin illness, internal and external parasites, bacterial infection, hypertension, inflammation, diabetes and cancer. *A. muricata* have been characterized as an anti-microbial, anti-inflammatory, anti-oxidant, cytotoxic to tumour cells. Clinical studies support the hypoglycemic activity of ethanolic extract of *A. muricata* leaves. Mechanisms of action of some pharmacological activities have been elucidated such as anti-oxidant, cytotoxic, anti-microbial, antinociception and hypotensive activities. The clinical studies are necessary to support the therapeutic potential of this plant. (Coria-Tellez *et al.*, 2018). The species of *Morinda* especially *M. citrifolia* has been

reported to have a broad range of health benefits for cancer, infection, arthritis, asthma, hypertension, and pain (Whistler, 1992). The roots, stems, bark, leaves, flowers, and fruits of Noni are all involved in various combinations almost 40 known and recorded herbal remedies (Bruggnecate, 1992). Additionally the roots were used to produce a yellow or red dye for tapa clothes and fala (mats), while the fruit was eaten for health and food (Aragones *et al.*, 1997).

Allium cepa root tip meristems have been widely used for the evaluation of cytotoxicity, anti-mitotic activity and genotoxicity by employing the growing roots of *Allium cepa* L. Root meristematic cells of *Allium cepa* L. have been used extensively in screening of drugs to evaluate their anti-mitotic activities (Anjana & Thoppil, 2013). *Allium* assay has been shown to have correlation with test in other living systems and serve as an indicator of toxicity of the tested material (Fiskesjo 1985). *Allium* test is sensitive test that has often been used for the determination of cytotoxic and genotoxic effects if various substances (Grant, 1982, Smaka *et al.*, 1996). All these peculiarities make it potent plant in the field of cytogenicity studies. Objective of this study was to investigate the effect of various concentration *Nyctanthes arbor-tristis* L. leaf extract on the root cells of *Allium cepa* L. *Annona muricata* and *Morinda citrifolia* leaf extracts were used in the in the present study to analyse the mitodepressive effect to understand the concentration dependent variation in the mitotic index of the *Allium cepa* root tip and also to compare the



mitotic index of control with the treated and to find out how much variation occurred in the mitotic index.

2. Materials and Methods

2.1. Plant materials selected for the present study

The plants selected for the present study includes *Annona muricata* L. (Annonaceae) and *Morinda citrifolia* L. (Rubiaceae). These two plants are having potential medicinal properties such as anti-microbial, anti-inflammatory, anti-oxidant, cytotoxic to tumour cells etc.

2.2. Methodology

Healthy and equal-sized bulbs chosen from a population of the common onion *Allium cepa* L. ($2n = 16$), were used as study material. These are considered one of the best biological models for the study of cytotoxicity. Before starting the experiment, the scales of the bulbs and the brownish button plate were removed, and the ring of the root primordial was left intact. The bulbs were allowed to germinate roots by soaking them in trays filled with moistened cotton. Once the root tips were initiated it is transferred to the experimental chemical at different concentrations taken in test tubes.

Fresh leaves of *Morinda citrifolia* and *Annona muricata* were collected, washed and then drained. Leaves were grinded in a mixer grinder and then it is filtered to obtain the extract. The concentrations of leaf extract taken for the present study was 5%, 10 % and 25 %. Each concentration taken in triplicate. The root tips treated in distilled water was used as control.

After the 24 hour treatment, the root tips of experimental samples were thoroughly washed in distilled water. Chromosome preparations were made using squash technique.

2.3. Methods of Squash Preparation

Cut the tip 5 to 8 mm from the tip of the freshly sprouted root. Then we discarded the rest of the root and washed them in water on a clean microscope slide. Placed one drop of 1N HCL on the root tip for making the root tip soft and added 2-3 drops of acetocarmine stain to the slide. Then the slide warmed gently over the alcohol lamp for about one minute.

Then a cover slip mounted on it avoiding air bubbles. It is carefully blotted on the excess stain with a blotting paper. The slide is squashed with our thumb using a firm and even pressure. It is observed under Olympus CX21i microscope in 10X objective. The region containing dividing cells are scanned and narrowed down and switched to 40 X for a better view. Total number of cell and the number of dividing cells were counted and mitotic index were calculated. Microphotographs were taken using Industrial Digital Camera UCMOS05100KPA.

3. Results and Discussion

The mitotic index is the ratio between the number of cells in mitosis and the total number of cells in a tissue sample. Mitotic indices are used as diagnostic tools in cancer treatment. Calculating a mitotic index is as simple as counting the number of cells in the sample that are in mitosis and also counting the total number of cells in

the sample. The mitotic index of onion root tips were calculated using the formula

$$\text{Mitotic Index percentage} = \frac{\text{No. of dividing cells}}{\text{Total no. of cells}} \times 100$$

Mitotic Index of the onion root tip cells in different concentrations of *Morinda citrifolia* treatment solution is analysed in the tables 1, 2 and 3 and that of control is given in Table 4.

Table 1. Treatment concentration *Morinda citrifolia* 5 %

Plane	Total No. of cells	No. of dividing cells	Mitotic index (%)	Average Mitotic index Percentage
A	102	5	4.902	5.844
B	91	4	4.395	
C	85	7	8.235	

Table 2. Treatment concentration *Morinda citrifolia* 10 %

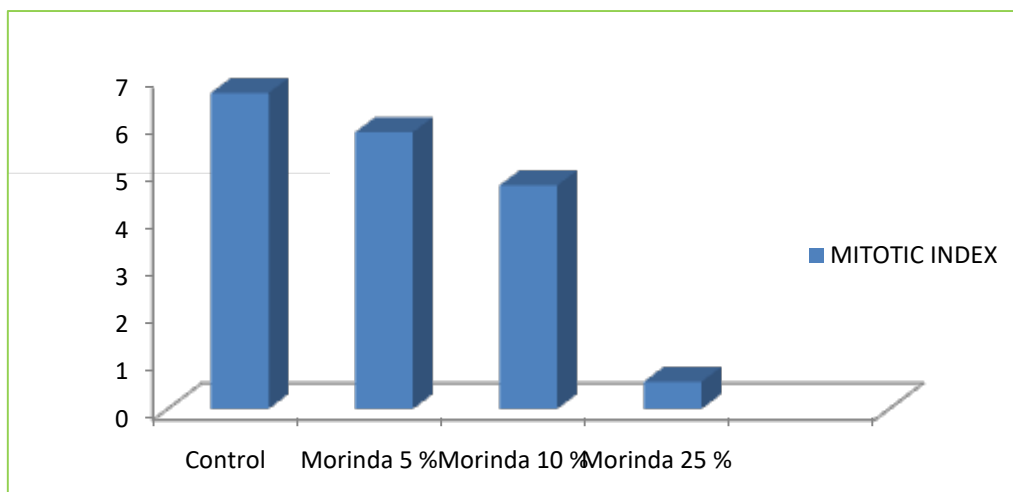
Plane	Total No. of Cells	No. of dividing cells	Mitotic index (%)	Average Mitotic index
A	46	3	5.299	4.721
B	29	2	6.883	
C	83	2	1.983	

Table 3. Concentration *Morinda citrifolia* 25 %

Plane	Total No. of cells	No. of dividing cells	Mitotic index (%)	Average Mitotic index (%)
A	105	2	1.255	0.574
B	142	1	0.468	
C	36	0	0	

Table 4. Control

Plane	Total No. of cells	No. of dividing cells	Mitotic index (%)	Average Mitotic index
A	136	7	5.147	6.674
B	96	8	8.333	
C	107	7	6.542	



Graph 1: Comparison of Mitotic indices (*Morinda citrifolia* Leaf extracts)

Mitotic Index of the onion root tip cells in different concentrations of *Anonna muricata* treatment solution is

analysed in the tables 4, 5 and 6 and that of control is given in Table 4.

Table 4. *Annona muricata* concentration 5%

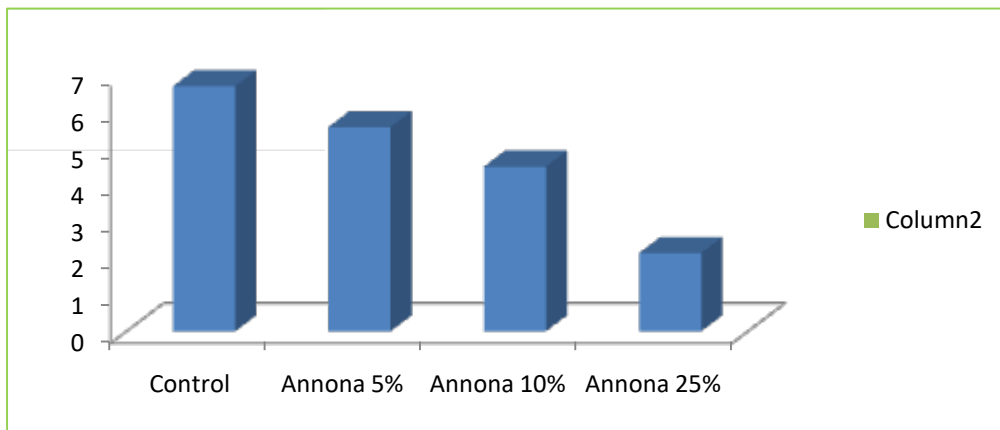
Plane	Total No. of cells	No. of dividing cells	Mitotic index (%)	Average Mitotic index (%)
A	66	4	6.060	5.562
B	93	5	5.497	
C	79	4	5.131	

Table 5. *Annona muricata* concentration 10 %

Plane	Total No. of cells	No. of dividing cells	Mitotic index (%)	Average Mitotic index (%)
A	82	3	3.256	4.484
B	80	4	4.502	
C	56	3	5.694	

Table 6. *Annona muricata* concentration 25 %

Plane	Total No. of cells	No. of dividing cells	Mitotic index (%)	Average Mitotic index (%)
A	51	2	3.930	2.131
B	81	1	0.876	
C	47	0	1.587	

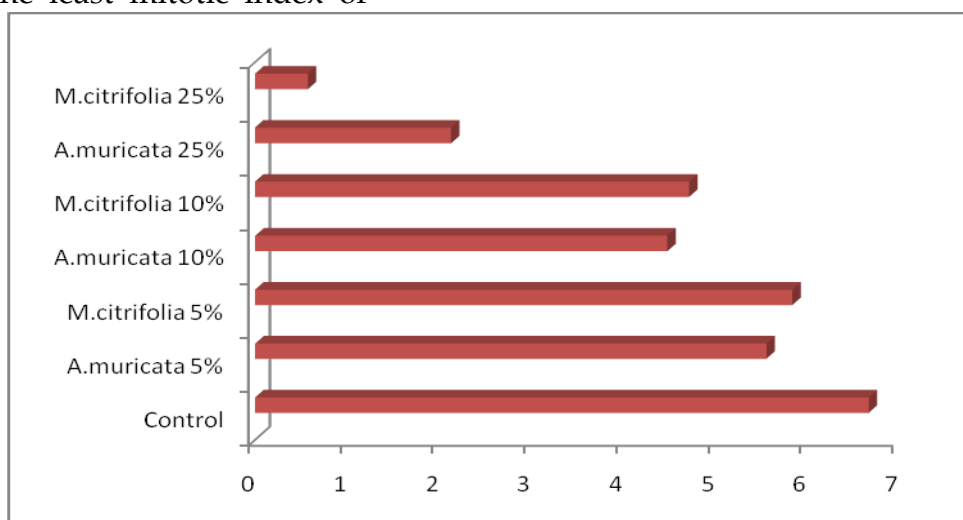


Graph 2: Comparison of Mitotic indices (*Annona muricata* leaf extracts)

Cells treated with leaf extract with higher concentrations had reduced mitotic indices compared with cells treated with water which was indicative of mito-depressive effect on the cell division of *Allium cepa* and *Morinda citrifolia*. The highest dose of leaf extract of *Morinda citrifolia* 25% solution shows the most mito-depressive effect with the least mitotic index of

0.574. In the case of *Annona muricata* 25% leaf extract solution shows the most mito-depressive effect with the least mitotic index of 2.131.

It is also observed that the length of the root is highly reduced in the treatment of leaf extract concentration 25%, in 10% it is medium sized and in 5% it is almost comparable to the control.



Graph 3: Comparison of Mitotic indices of different concentrations of leaf extracts of *Annona muricata* and *Morinda citrifolia*.

PLATE 1



A



B



C



D



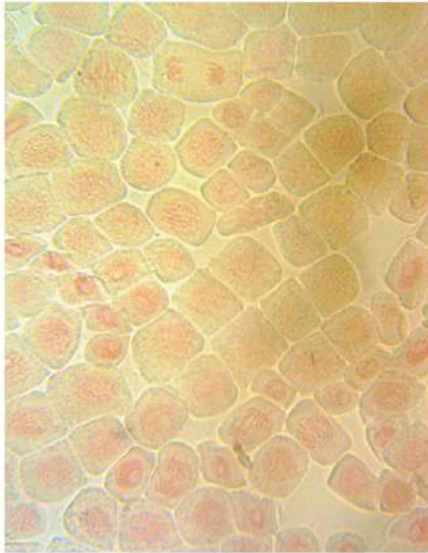
E

A. MORINDA CITRIFOLIA B. PREPARATION OF LEAF EXTRACT

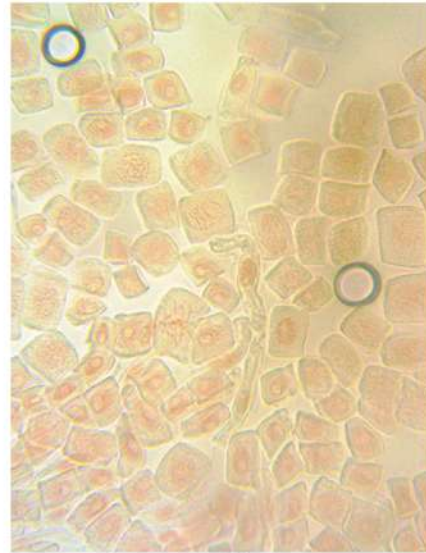
C. ONION ROOT TIPS IN TREATMENT D. SLIDE PREPARATION

E. OBSERVING AND TAKING MICROPHOTOGRAPHS.

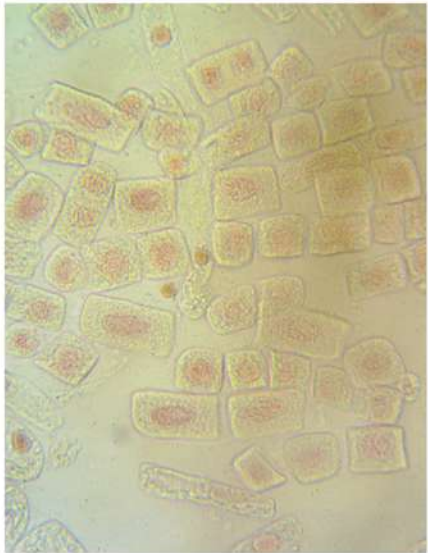
PLATE 2



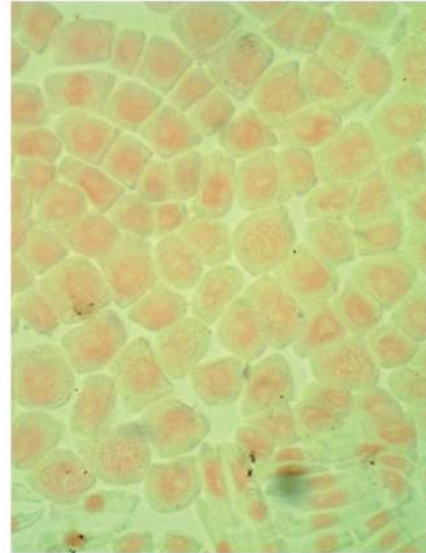
A



B



C



D

A. CONTROL B. TRATMENT CONCENTRATION 5%

C. TRATMENT CONCENTRATION 10%

D. TRATMENT CONCENTRATION 25%

PLATE 3



A



B



C



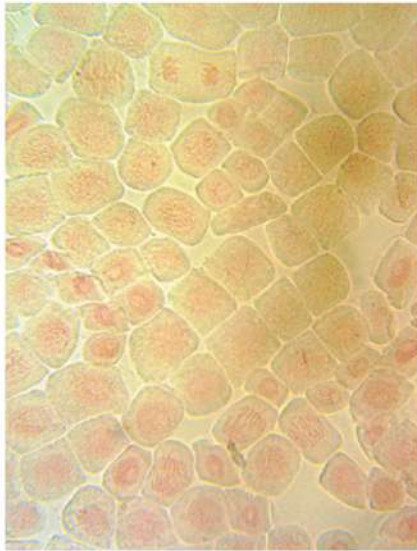
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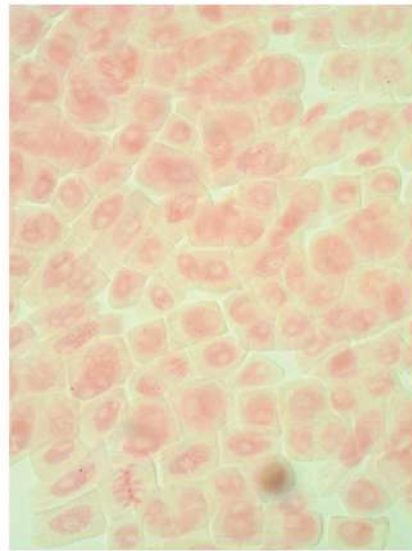
E

A. ANNONA MURICATA B. PREPARATION OF LEAF EXTRACT
C. ONION ROOT TIPS IN TREATMENT D. SLIDE PREPARATION
E. OBSERVING AND TAKING MICROPHOTOGRAPHS.

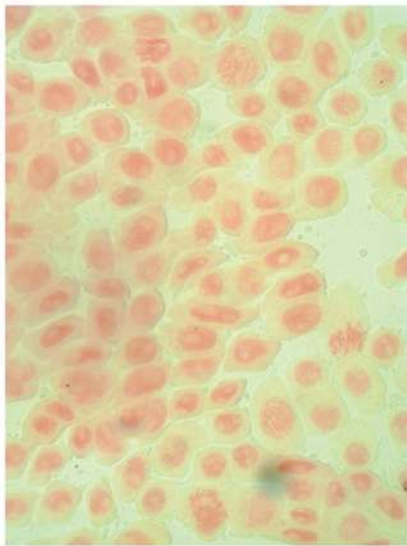
PLATE 4



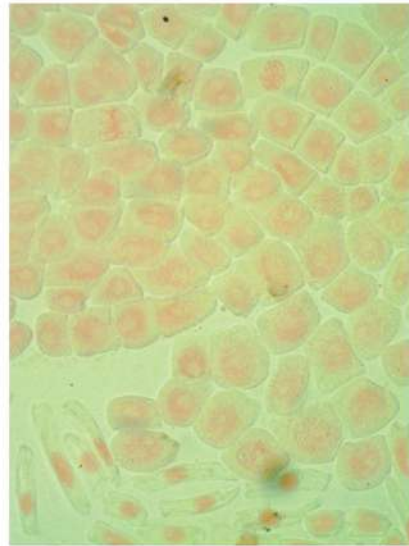
A



B



C



D

A. CONTROL B. TRATMENT CONCENTRATION 5%

C. TRATMENT CONCENTRATION 10%

D. TRATMENT CONCENTRATION 25%

4. Conclusion

The cytological observations revealed that with increasing concentrations of leaf extract, the mitotic index reduced to variable degrees. Mitotic index decreased in a dose dependent manner as is evident from observations recorded in table 1 to table 7.

The Graph 1 and 2 exhibited pronounced reduction in mitotic index at the dose level of 25%. The decrease in Mitotic Index values in the treated onion roots is an indication of cytotoxic substances in leaf extract which cause inhibition of mitotic activities. The reduction in length of the roots indicates the growth is inhibited by this phytochemical.

The result of the present study suggests the potential use of *Annona muricata* and *Morinda citrifolia* as a therapeutic agent. The mitotic depressive effect induced by these plant extracts suggests that it has some effect on cell division of *Allium cepa* L. which may be due to the condition induced by the chemical components of the extracts. These plants can be used in the treatment of uncontrolled cell divisions because it can control cell division in appropriate concentrations. Further studies have to be conducted to isolate the specific phytochemical which give the leaf extract with the specific activity of mitotic depression.

5. References

- Abubakar, M.S., Musa, A.M., Ahmed, A. and Hussaini, I.M. (2007).** The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of Northern Nigeria. *Journal of Ethnopharmacology*, **111(3)**: 625-629.
- Aragones, E.G., Magpayo, F.R. and Foronda, S.U. (1997).** Three economically important dye plants in Southern Philippines. *Journal of Science*, **23**: 87-95.
- Auti, S., Pagare, R., Hire, D. and Saawale, V. (2010).** Economically important plants in Southern Parts of Africa. *Journal of Cell Tissue Research*, **10(3)**: 2331-2335.
- Bruggnecate, J.T. (1992).** Native plants can heal your wounds. Honolulu Star-Bulletin Local News.
- Coria-Téllez, A.V., Montalvo-González, E., Yahia, E.M. and Obledo-Vázquez, E.N. (2018).** *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity *Arabian Journal of Chemistry*, **11(5)**: 662-691.
- El-Shabbaby, O.A., Abdel Migid, H.M., Soliman, M.I. and Mashaly, I.A. (2013).** Genotoxicity screening of industrial waste water using the *Allium cepa* chromosome aberration assay. *Pakistan Journal of Biological Sciences*, **6**: 23-28.



- Emmanuel, C. (2015).** Traditional medicine and the future of plants in Nigeria. *Journal of Medicinal Plants Studies*, 3(4): 23-29.
- Fiskesjo, G. (1985).** The *Allium* test as a standard in environmental monitoring. *Hereditas*, 120: 99-112.
- Grant, W.F. (1982).** Chromosome aberrations assays in allium report of the USEPA gene programme. *Mutation Research*, 99: 273-291.
- Kuras, M., Nowakowska, J., Sliwinska, E., Pilarski, R., Ilasz, R., Tykarska, T., Zobel, A and Gulewicz, K. (2006).** Changes on chromosome structure, mitotic index and nuclear DNA content from cells of *Allium* test induced by bark water extract of *Uncaria tomentosa* (Willd.) DC. *Journal of Ethnopharmacology*, 107: 211-221.
- Smaka-Kinci, V., Stegnar, P., Lovka, M. and Toman, M.J. (1996).** the evaluation of waste, surface and ground water quality using the *Allium* test procedure. *Mutation Research*, 36: 171-179.
- Soetan, K.O and Aiyelaagbe, O.O (2009).** The need for bio activity safety evaluation and conservation of medicinal plants. *Journal of Plants Research*, 3(6): 324-325.
- Whistler, W. (1992).** Tongan herbal medicine. *Isle Botanica*, Honolulu Hawaii, pp89-90.