RESEARCH ARTICLE

ISSN: 0331 - 670X
https://doi.org/10.51412/psnnjp.2021.14

The Nigerian Journal of PHARMACY
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THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

Cytotoxicity, genotoxicity and nutritional compositions of ethanolic extract of Ficus *capensis* leaves

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ARTICLE INFO

Article history:

Received	21 April 2021
Revised	11 June 2021
Accepted	3 July 2021
Online	30 Sept 2021
Published	-

Keywords:

Genotoxicity;
Allium cepa;
Ficus capensis;
chromosome aberrations

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ABSTRACT

Background: *Ficus capensis* leaves extract has been used in traditional medicine over the years by Middle belt ethnic group in Nigeria to treat a wide range of diseases and also serve as food.

Materials and Methods: Genotoxic potentials, cytotoxic effects and nutritional composition of the ethanol extract of the leaves were investigated using *Allium cepa* assay and proximate analysis respectively. The *Allium cepa* mitotic inhibitory activities of the extract at varying concentrations of 10mg/mL, 20mg/mL, 50mg/mL and 100 mg/mL was investigated. Methotrexate and Distilled water were considered as standard drug and control respectively. All experiments were carried out in triplicates.

Results: Proximate analysis result revealed crude fibre, carbohydrates and proteins as the imost abundant macro nutrients with percentage composition of $37.2 \pm 0.08\%$, $30 \pm 0.02\%$ and $14.70 \pm 0.08\%$ respectively. Also, a significant ($P<0.05$) dose dependent decrease in mitotic index of the extracts as compared to the control was observed. Chromosomal aberrations scored were stickiness, lagging, bridges, vagrant chromosome and micro-nucleated interphase cells were also observed.

Conclusion: This study confirmed that although the ethanol leaves extract of *Ficus capensis* is a good dietary source of carbohydrates, crude fibre and proteins, it exhibits significant mito-depressive and genotoxic effects at all the tested dosage.

1. Introduction

Medicinal plants play a significant role in healthcare as alternative source of drugs for majority of populations that are faced with inadequate supply of orthodox drugs and facilities in various parts of the world including Nigeria.[1,2]
Aside from bioactive compounds that are reported to be present in medicinal plants, there exist phytonutrients, which play important roles in providing energy to power active life processes as well as serving as precursors of biomolecules involved in essential life processes, thereby promoting good health. [3]
In addition to bioactive compounds that are reported to be present in medicinal plants, there exist phytonutrients, which play important roles in providing energy to power active life processes as well as serving as precursors of biomolecules involved in essential life processes, thereby promoting good health.[3]
Aside their dependability as source of food as well as medicine for treating ailments, increasing number of medicinal plants have been reported to exhibit cytotoxic and genotoxic properties. [4, 5, 6] However, the presence of

cytotoxic and genotoxic substances in the herbal preparation can impair health if such plants are consumed by humans.[7] Genotoxic effect of medicinal plants manifests as chromosomal structural alteration through cytogenetic analysis.

According to Parra *et al*. [8], an investigative study on pharmacological activities of medicinal plants must also take into consideration, the toxicological screening of the plants in order to ensure its safety.[8]

Ficus capensis, popularly known as bush fig of heaven, is a fast growing, deciduous or evergreen trees, usually grow to about 5 - 12 m in height but may attain a height of 35 - 49 m.[9] In Nigeria, the tree is cultivated in all parts of the country but abundant in the middle-belt (North- Central) of Nigeria. *Ficus capensis* has been used extensively for the treatment of leprosy, epilepsy, rickets, infertility, gonorrhea, oedema, respiratory disorders and dysentery in varying communities in Nigeria. Several studies have shown that the plant exhibits some pharmacological properties including anti-bacterial, anti-trypanosomal, anti-diarrhea, anti-ulcer, antioxidant and anti-sickling activities, all of which justified its ethno medicinal applications by the people in these areas.[10,11,12,13,14]

However, there is paucity of information about its cytotoxicity and genotoxicity. Therefore, this study is aimed at screening ethanol extract of *Ficus capensis* leaves for its cytotoxic and genotoxic activities as well evaluating its nutritional significance.

2. Materials and methods

2.1 Chemicals and reagents

Reagents used for the study were distilled water, methotrexate drug, orcein stain and analytical grade chemicals obtained from Sigma-Aldrich, Burlington headquarters, USA, which include ethanol, anthrone, hydrochloric acid and acetic acid.

2.2 Plant collection and identification

Ficus capensis ("Opoto") leaves was purchased from Mushin market, Lagos State (6.5273^{0}N, 3.3414^{0}E). The plant sample was authenticated and given a voucher number LUH 1210A in the herbarium section of Botany department, Faculty of Science, University of Lagos, Lagos state. The leaves of *Ficus capensis* were shade dried for approximately 48 hours and ground using a laboratory hammer mill.

2.3 Extraction of plant material

The ground dried leaves was weighed using weighing balance and its weight was recorded to be eight hundred (800g). Eight hundred grammes (800g) of the grounded leaves was extracted with 96 - 99% ethanol solvent using Soxhlet extractor for about 18 hours. After which, the solvent extract collected was concentrated on a water bath set at 80^{o}C to evaporate solvent from the extract. Thereafter, the sample concentrate obtained was carefully washed with distilled water several times to remove remnant ethanol in the extract which was eventually eliminated using a freeze dryer. The percentage yield of *Ficus capensis* solvent extract was determined using the Equation 1

$$\% \text{ Yield} = \frac{\text{Weight of the dried extract} \times 100}{\text{Weight of the leaves sample}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\text{Equation 1}$$

2.4 Preliminary phytochemical tests

Phytochemical analysis was carried out using standard procedures (15-16).

2.5 Quantitative Phytochemical analysis

Total flavonoids and tannin contents of the grounded dried *Ficus capensis* leaves sample were determined spectrophotometrically as described by Trease and Evans [17] while quantitative determination of Saponin content of the plant sample was carried out according to the method of Obadoni and Ochuko.[18]

2.6 Proximate analysis

The grounded leaves of *Ficus capensis* were analyzed for their percentage moisture content, total carbohydrate, crude protein, lipids, crude fibre and ash contents according to the AOAC.[19]

2.7 *Allium cepa* assay

Cytotoxic and genotoxic activities of *Ficus capensis* leaves extracts on *Allium cepa* meristematic cell growth and chromosomal structure were determined using *Allium cepa* assay as described by Shrivistava *et al.* [20]

Allium cepa bulbs (Onion) of medium and uniform size bought from Oyingbo Local market (6.4790^{0}N, 3.3918^{0}E) of Lagos State were shade dried for one week to reduce the moisture content and facilitate root growth. Bulbs were carefully scrapped with 100 Tiger carbon double edge razor blades to remove the outer dried skins and dried meristematic roots, after which they were suspended on a set of sterile universal bottles filled with distilled water for 48 hours at ambient temperature such that their base were in contact with water in each of the universal bottles and then incubated in the dark for 48 hours. After 48 hours of incubation, when the rootlets had emerged, bulbs with root tips grown to 2-3 cm were selected for the study. The selected bulbs were placed in a set of universal bottles filled with *Ficus capensis* ethanol extract solution of different concentration, 10mg/mL, 20mg/mL, 50mg/mL and 100mg/mL. The concentrations tested in the study were considered according to the established LD_{50} of *Ficus*

capensis leaves extract as reported by Njoku *et al*. [21] Meanwhile, Bulbs sprouted in water and methotrexate were used as control and standard drug respectively for the purpose of comparison.

All experiments were set up in triplicates and the entire set up were incubated at 22 ± 2°C for 72 hours away from direct sunlight. Thereafter, the root tips from each of the bulbs were rinsed with distilled water, carefully cut and preserved in fixative containing sample container. Root tips squash preparations, subsequent staining with lactic acid-orcein stain followed by 20 minutes incubation of the stained root tips smear were carried out on microscope slides for microscopic studies. Thereafter, numbers of dividing and non-dividing cells as well as total number of cells from four microscopic fields were counted using bright field light microscope, after which the mitotic index was calculated as described by Shrivistava *et al.* (20) using the Equation 2

$$\text{Mitotic Index} = \frac{\text{Number of Dividing cells}}{\text{Total number of cells}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\text{Equation 2}$$

Dividing cells = number of cells in prophase + number of cells in metaphase + number of cells in anaphase + number of cells in telophase.

The Genotoxic and cytotoxic effects of the leaf extract solution were determined according to the method described by Tulay and Ozlem, [7] by observing chromosomes for structural aberrations revealed from photomicrographs and reduction of rate of mitosis of *A. cepa* root meristematic cells sprouted in different concentrations of the plant leaves' extract solution as compared to the control respectively.

2.8 Statistical analysis

The mitotic indexes obtained as well as calculated percentage yield of *Ficus capensis* leaves extract were expressed as mean± standard error of mean (S.E.M) using SPSS 14.0 version computational software and the significant differences in the mitotic index across various treatments were tested using One-way Analysis of variance (ANOVA) with the level of significance considered at $P< 0.05$.

3. Results

Phytochemical analysis revealed the presence of phenols, tannins, phlobotannins, Saponin, Anthraquinone, cardiac glycosides, glucose, and terpenoids in ethanol extracts of *Ficus capensis* leaf. However, alkaloids and steroids gave negative results with phytochemical test, which implied that they are absent in *Ficus capensis* leaf sample analyzed as revealed in the Table 2.0 below. Also, quantitative determination of flavonoid and tannin contents of the plant extracts revealed the % composition of flavonoid and tannin in the plant sample analyzed to be 2 .84% and 6.03% respectively as shown in the Table 3.0 below.

The proximate analysis conducted on *Ficus capensis* leaves extracts revealed higher percentage composition for crude fibre (37.2±0.08%), total carbohydrates (30±0.02%) and crude protein (14.7±0.08%) as compared to other nutritive components found in the leaf of *Ficus capensis* plant sample as presented in the Table 3. below. However, Fat/Lipid has the least percentage abundance of the nutritive components with % composition of 1±0.08%.

The result of *Allium cepa* analysis was presented in form of mitotic index values of Onion bulb roots sprouted in *Ficus capensis* leaves extracts of different concentrations. The result obtained showed that ethanol extract of *Ficus capensis* leaf sample solutions at all concentrations tested exhibits significant mito-depressive effect on *Allium cepa* meristematic root cells when compared with control as indicated in their mean mitotic index of meristematic cells from roots sprouted in different concentrations of plant leaf ethanol extract and water. However, dose dependent significant decrease of Mean mitotic index of meristematic cells in Onion bulbs grown in the different concentrations of the plant extract solution was observed from the finding shown in Table 4.0. Meanwhile, there was no significance difference in the mean mitotic index of cells sprouted in methotrexate and highest concentration (100mg/ml) of *Ficus capensis* leaves extract solution.

Photomicrographs of meristematic cells from bulb roots sprouted in different concentrations of *Ficus capensis* leaves extract solution revealed occurrence of chromosome structural aberrations such as chromosome bridge, sticky and clumpy chromosomes as well as vagrant chromosomes at various stages/phases of cell growths and these are presented in the fig.1 shown below.

Contrarily, rapidly dividing meristematic cells from onion bulbs grown in water showed normal mitosis at different stages of cell division with no evidence of chromosomal aberrations as revealed in the Figure 2 below.

Table 1 Percentage yield of *Ficus capensis* leaves extract.

Plant sample	Percentage yield (%)
Ficus capensis	15.5±0.12

Table 2 Qualitative phytochemical Analysis of *Ficus capensis*

Phytochemicals	*Ficus capensis*
Phenols	++
Tannins	++
Saponins	++
Phlobotannins	++
Cardiac glycosides	++
Anthraquinones	++
Terpenoid	++
Steroid	- -
Alkaloids	- -
glucose	++

++ Indicates the presence of the phytochemical.

- - Indicates the absence of the phytochemical.

Table 3 Proximate Analysis of *Ficus capensis* plant

Nutrient	% composition.
Moisture	6.22±0.02 %
Ash	6.29±0.06%
Crude fibre	37.2±0.08%
Total protein	14.7±0.08%
Total carbohydrate	30±0.02%
Fats	1±0.08%

Table 4 The mean and standard error of the mean mitotic index (%) of onion bulb cells sprouted in various concentrations of ethanol extract of *Ficus capensis* leaves including the controls.

Plant Extract	N	Mean ± standard error of mean
Ficus capensis 10mglmL	*4*	3.5925 ± 0.56998^{c}
Ficus capensis 20mglmL	*4*	0.66566 ± 0.2159^{b}
Ficus capensis 50mglmL	*4*	0.2800 ± 0.28000^{c}
Ficus capensis 100mglmL	*4*	0.0000 ± 0.0000^{a}
Control	*4*	14.9175 ± 5.8700^{d}
Methothrexate (125mg/mL)	*4*	0.0000 ± 0.0000^{a}

*$P < 0.05$. Treatments with difference superscripts indicate significance difference

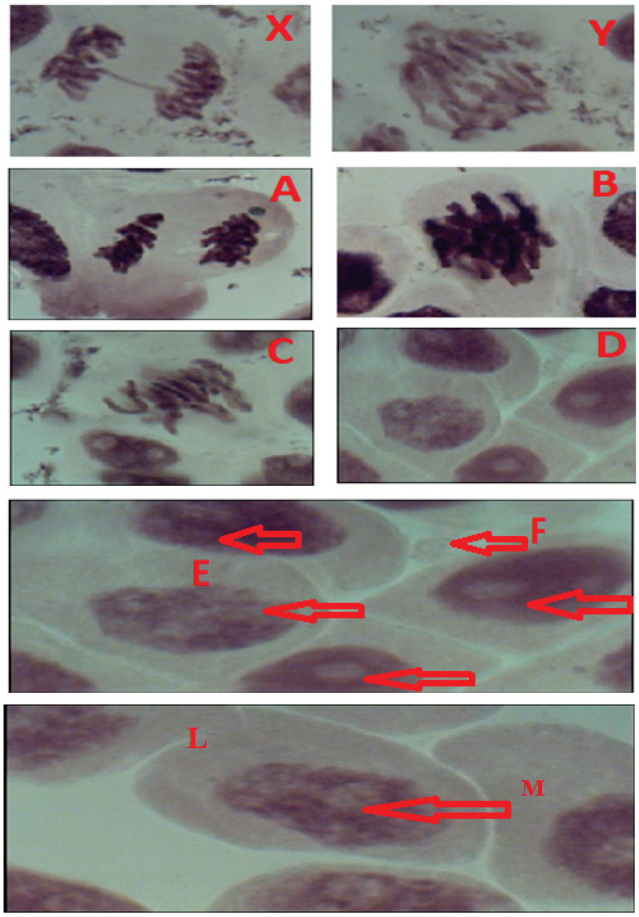

Fig 1: Photomicrographs of onion's meristematic cells grown in different dosages of *ficus capensis* solutions and methrotrexate respectively.

Arrows shown in the cell X and Y indicate occurrence of Chromosome Bridge in the anaphase cells X and Y from bulb root grown in 10mg/mL *Ficus* solution. Arrows in the cells A and B revealed sticky and clumpy chromosomes in the cells at anaphase and metaphase phase from the roots sprouted in 20mg/mL *Ficus* solution.

Cells in Figure 1C and D from roots sprouted in 50mg/mL *Ficus* solution were at metaphase and interphase phase of mitosis. Presence of vagrant chromosomes and improper alignment of chromosomes on the metaphase plates were indicated by the arrows in cell 1C while vacuolated interphase cells were evident in cell 1D as indicated by the arrow.

Also, in Figure 1E and F from bulb roots grown in 100mg/mL *Ficus* solution were at interphase with the nuclear membrane of cells in Figure 1E gradually deteriorated, while F represents vacuolated cells at interphase stage of mitosis.

In the same way, cells in Figure 1L and M from onion bulb roots sprouted in 125g/mL of Methotrexate solution were vacuolated and none dividing as indicated by the arrows .

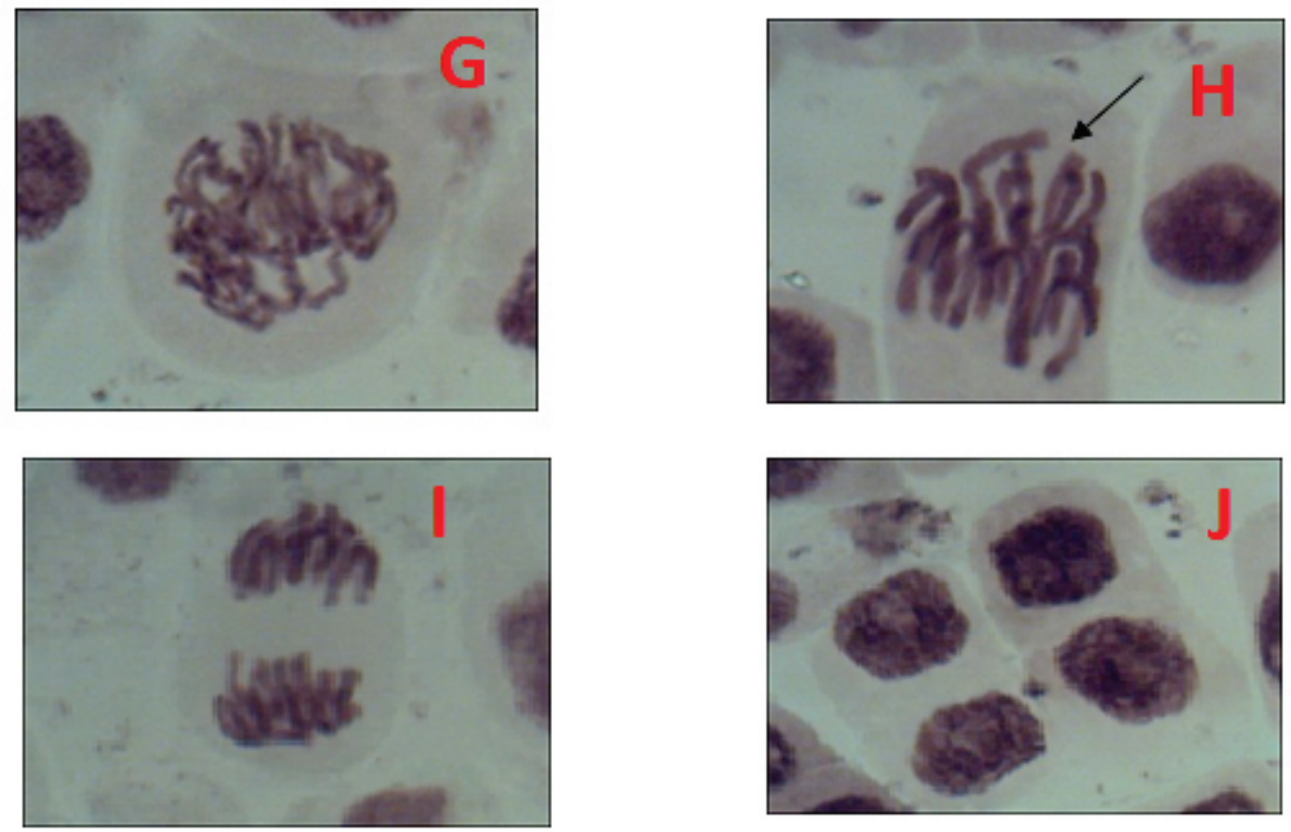

Fig 2: Photomicrographs of *Allium cepa* meristematic root cells G-J grown in water (control). Cells were at different stages of mitosis. cells G-J were at prophase, metaphase, anaphase and telophase respectively with no chromosomal abberration observed**.**

4. Discussion

Nutritive values of medicinal plants are assessed by quantification of their phytonutrient's constituents by proximate analysis, which enables humans to appreciate the roles they play in essential life processes and general well-being of the body. The leaves of *Ficus capensis* plant is more abundant in crude fibre, total carbohydrate and crude protein as compared to other nutritive components in the leaves. However, crude fibre was shown to be the most abundant of all the macro nutrients found in *Ficus capensis* leaves. The estimated values of crude fibre and carbohydrates in this study corroborate with the work of Adebisi and Oyeleke [22] who reported 37.3% and 30.93% for crude fibre and carbohydrates, respectively. However, the percentage crude protein content (14.7%) of *Ficus capensis* leaves obtained in this study is in contrary to the findings of Odesanmi *et al*. [23] who reported lower value of crude protein content of ethanol extracts of bark and leaves of *Ficus capensis.*

Variations in the values of nutritive components of *Ficus capensis* leaves reported by several nutritional studies [11,14,23-25, 26-27] as well as the present study have been widely observed. The discrepancy in the results obtained from various studies could be due to different locations of cultivating the plant, failure to carry out the methodology without modification as well as failure to use analytical grade reagents and materials for the study. Thus, it is evident from the study that *Ficus capensis* leaves is highly rich in carbohydrates, protein and crude fibre and can serve as better source of these essential nutrients for proper functioning of the body system.

The preliminary phytochemical studies indicated of the presence of tannins, anthraquinones, phlobotanins, sugars, flavonoids, cardiac glycosides, saponins, terpenoids and phenols. The absence of alkaloid as reported in the present study is in agreement with the findings of Ayinde and Owolabi [9] who determined the effects of the aqueous extract of *Ficus capensis* leaves on gastro-intestinal motility. Whereas, this contradicted the results of Obong *et al*. [28] who reported the presence of alkaloid and steroids in a study conducted on comparative phytochemical, antioxidant and antimicrobial properties of *Ficus capensis*, *Aristolochia ringens*, *Albizia zygia* and *Lannea welwitscii*. Furthermore, quantitative phytochemical analysis showed saponin as the major phytocompound in the plant leaves. The presence of the secondary metabolites in medicinal plants probably explains the various uses of the plants for traditional medicines. Saponin has been known to exhibit anti-tumourigenic effects through varieties of antitumor pathways.[29] Hence, high amount of saponin content in *Ficus capensis* suggests that the plant may be effective for cancer treatment.

Moreover, finding from this present study showed that ethanolic leaf extract of *Ficus capensis* plant exhibit significant ($P< 0.05$) dose dependent anti-mitotic effects on *Allium cepa* root cell division. The dose dependent mito-depressive effects observed in the present study is in agreement with the report of a study conducted by Shrivastava *et al*. [20] on anti-mitotic and anti-proliferative activity of stem Bark of *Oroxylum indicum,* in which the plant extract was also observed to inhibit *Allium cepa* mitosis in dose dependent manner but with lower effect as compared to that of *Ficus capensis* extract. The previous study conducted by Shivasharanappa and Ramesh on clot lysis and anti-mitotic activities of *Ficus glomerata* showed that the methanol extract of the plant was highly effective in reduction of root length, root numbers, and mitotic index as compared to the control. However, the effect of dosages of the different solvent extracts of the plant leaves were not reported.

The dose dependent cytotoxic effect of the ethanolic extract of *Ficus capensis* leaves in this study could be attributed to variation in the quantity of cytotoxic phytocompounds present in the various concentrations of the ethanol extract of *Ficus capensis* leaves. The presence of such phyto-compounds may compromise the function of one or more cell cycle regulatory protein(s) essential for cell division and growth, thereby reducing the rate of cell division in the plant. However, dosage of the plant extract below 10mg/ml might not show cytotoxic activity since no information about its potency to exert cytotoxic effect has been reported. If such dosage when further tested shows no cytotoxic activities, could be a safe dosage to be considered for herbal therapy.

Also, several chromosomal aberrations noted from root cells sprouted in various concentrations of the plant extract including vagrant chromosomes, chromosome bridges, sticky and clumpy chromosomes observed from the photomicrographs are strong evidence to show that all the tested dosages of the plant extract exhibit genotoxic potential on *Allium cepa* cells. However, similar chromosomal aberrations of *Allium cepa* root cells were reported for stem Bark extract of *Oroxylum indicum* in anti-mitotic study of the plant part conducted by Shrivastava *et al*. [20]

Although the present study revealed the cytotoxicity and genotoxicity exhibited by *Ficus capensis* leaves extract, the cytotoxicity study conducted in the study was tested only on plant cells and tissues (*Allium cepa*), which is the major limitation of the study. To be sure of its broad-spectrum cytotoxicity and genotoxicity properties, further *in vivo* and *in vitro* cytotoxicity study involving animal and human cells/tissues need to be conducted for thorough screening of the plant leaves, in order to ascertain its safety for human consumption.

5. CONCLUSION

Inconclusion, *Ficus capensis* leaves is rich in carbohydrates, crude fibers and crude proteins. However, genotoxic and cytotoxic potentials of the leaves extracts at all dosages tested were evident in form of chromosomal aberrations and its Mito-depressive effects on *Allium cepa* meristematic cells respectively.

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