

Phytochemical Screening and the Mutagenic Effect of *Physalis Angulata* (L.) Plant Extract Using *Allium Cepa* (L.) Root Tip

¹Waja Samuel, M. M. Malgwi² and S.Y. Simon³

¹Department of Biological Science Tech. Federal Polytechnic Mubi, Adamawa State, Nigeria. ²Department of Plant Science, Madibbo Adama University Yola, Adamawa State, Nigeria. ³Department of Crop Production, Madibbo Adama University Yola, Adamawa State, Nigeria.

Corresponding author: samuelwaja@rocketmail.com

Abstract

In this study, phytochemical screening and the assessment of the cytotoxic and mutagenic ability of *Physalis angulata* plants extracts using *Allium cepa* root tip was evaluated, and aqueous plant extract of *P. angulata* was prepared by soaking 100 grams of the dried and pounded plants material in 1 liter of water for 72 hours and was filtered using Whitman No 1, then subjected to qualitative phytochemical screening for saponins, tannins, alkaloids, flavonoids, phytosterols, glycosides, and terpenoids. for cytological analysis, *A. cepa* root tip was harvested pre-sorted in 0.05% colchicine for five hours for pretreatment then fixed in 1:3 v/v glacial acetic acid for 24 hours then hardened in absolute ethanol, the root tips were then hydrolyzed in 1NHCL for 8-10 minute, the chromosomal aberrations observed were polyploidy, dissolution, vagrant metaphase, binucleate, laggard, nuclear lesion, fragmentation, chromosome gap, anaphase bridge, micronuclei, unequal separation. The differences in the percentage of aberrations were analyzed statistically using ANOVA, the result shows a significance different between the percentage of chromosomal aberrations on the root tip cells of *A. cepa* treated with *P. angulata* leave extract, 5g (P =0.122) and 15g (P = 0.122), but 15g is significantly different from 5g (P =0.003), but not statistically different from 10g (P =0.122). 20g is significantly different from 5g (P =0.000), and 10g (P = 0.002), but not statistically different from 15g (P = 0.097). a positive control is statistically different from 5g (P =0.000), 10g (P =0.005), and 15g (P =0.026), but not statistically different from 20g (P=0.549). for *P. angulata* fruits extracts 10g is significantly different from 5g (P =0.012), but not statistically different from 15g (P =0.161), 20g is significantly different from 5g (P =0.00) and 10g (P =0.002), but not statistically different from 15g (P =0.081) and positive control (P =0.638) As the concentrations of the plant extracts increased to 10 %, 15 %, and 20 %, there was progressive increase in the percentage of aberrant cells and decrease in the number of dividing cells. Low concentration of the plant extracts and wide spacing of dosage is therefore suggested for use in the treatment of ailments.

Keywords: *Physalis angulata*, *allium cepa*, chromosome aberration, vagrant metaphase.

Introduction

Medicinal plants for a long period have been the first choice for the population in the area, and studies conducted in many parts of African countries have shown that some people treated with various medicinal plants presented adverse effects (Tchacondo *et al.*, 2011). Medicinal Plants are a source of innovative drugs, including those used in pharmaceuticals, nutraceuticals, food supplements, (S. Wilson, & O. A. W Handy, 2022), folk remedies,

modern medicine, pharmaceutical intermediate bioactive principles, and lead molecules in synthetic drugs (Praveen *et al.*, 2010; Tariq *et al.*, 2013). Secondary metabolites, which have been demonstrated to exhibit a variety of biological functions, are largely responsible for the scientific basis for the traditional use of plants in medicine in many ancient civilizations (Bourgaud *et al.*, 2001). Advances in Analytical methods like chromatography made it possible to recover more and more of these compounds, which eventually served as the foundation for the field of phytochemistry. (Hussein and El-Anssary, 2018). Some of the phytochemicals to which the protective and therapeutic benefits of plants have been attributed include alkaloids, terpenoids, tannins, and phenolic among others (Harborne, 1998; Zhang *et al.*, 2001). (Stanley Wilson, & O. A W. Handy, 2022). Plants material such as leaves, fruits extract in general are rich in antioxidants such as flavonoids and phenolic compounds (Luximon-Ramma *et al.*, 2003). These phytochemicals were proven to be effective against various mutagens either through scavenging free radicals or by their reducing power, thus decreasing the dangers present by ROS to the genome (Akinboro *et al.*, 2011; Mazio and Soliman, 2009). Thus, it is believed that the healing property of phytochemicals was the implicit success of the use of plant-based raw materials in the well-known Indian medicinal systems, namely, the Ayurveda, Unani, and Siddha and most Chinese folk medicines (Jayaraj, 2010; Krishnaraju *et al.*, 2006).

However, it is very important to investigate the pros and cons of the plants to the human system. There are many bioassays available to evaluate the cytotoxicity and mutagenicity of various chemical compounds. Among them, higher plants are often used in many studies since they are not expensive, well correlated with other animal test systems, and easily available (Fiskesjö, 1985). The Allium test is regarded as a good bio-indicator test system due to its simplicity, affordability, ease of use, and good correlation with other test systems, according to the International Program on Plant Bioassay, US Environmental Protection Agency (EPA), World Health Organization (WHO), International Program on Chemical Safety, and United Nations Environmental Program. Allium test may assess a variety of parameters, including root growth, membrane integrity, antioxidant enzyme activities, MI, and CAs because of its quickly growing roots and its large and reduced (Bianchi *et al.*, 2016; Rosculete *et al.*, 2019; Liman, 2020; Macar, 2020; Maity *et al.*, 2020; Srivastava and Singh 2020; Liman *et al.*, 2021). The Allium cepa assay is a plant bioassay that has been used in vivo and has been validated by the international program on chemical safety (IPCS, WHO) as an effective and standard test for chemicals screening, in situ monitoring of the genotoxicity of environmental substances, and to assess the genotoxic potential of medicinal plants (Leme and Marin-Morales, 2009). (Camparoto *et al.*, 2002; Knoll *et al.*, 2006; Fachinetto *et al.*, 2007; Lubini *et al.*, 2008; Fachinetto *et al.*, 2009). The Allium cepa assay is also a short-term in vivo cytotoxicity test that tests cytotoxicity using chromosomes and therefore detects chromosome structural and numerical alterations (Tedesco and Laughinghouse, 2012; Bonciu *et al.*, 2018).

Physalis angulata (L.) popularly called golden berry is an exotic fruit that belongs to the *Solanaceae* family. The fruit originated in South America and today it is grown commercially

in several tropical and subtropical countries (deOliveira *et al.*, 2020). A golden berry is an annual plant that grows all over the world. Golden berries are popular fruits known for their organoleptic properties (flavor, odor, and colour) nutritional value (vitamins A and C, potassium, phosphorus and calcium, and health benefits (Puente *et al.*, 2011). The traditional usage of golden berries is numerous. It is named "kakanji" in the Iranian traditional medicine text (ITM) and it is used for liver, kidney, or bladder dysfunctions, and helminths disease (Anghili and Makhzan, 2001). However, when they are been prescribed by traditional healers, their dose-related cytotoxicity and mutagenic levels are not considered during prescription. The herbalist usually focus only on the ailment or disease and the appropriate dosage to give, and also very little is known about the phytochemical constituent of these important medicinal plants, though some patient may show some allergic reactions to some molecules present in their extracts (Amoah *et al.*, 2006) Also the dosage of the healing plant extract prescribed by the traditional healers may not be accurate, thus can become cytotoxic or mutagenic to the patient and cause adverse effects. Therefore, this research is aim at determining the phytochemical present in the leave and fruit extract of *Physalis angulate* and the mutagenic effects of *P. angulate* extracts on the root tip of *A. cepa*.

Material and Method

Sampling site for the medicinal plants (collection and authentication of plant samples)

Leaves of *Physalis angulate* were collected around Mubi North and Mubi South local government area of Adamawa state, all collected plants specimen was moved down to the department of plant science Modibbo Adama University for identification using the preserved specimen voucher. Each harvested plant material was given a code. The leaves of the collected medicinal plants were washed, dried, and store under shade in a greenhouse at the plant Science Department Modibbo Adama University, Yola Adamawa state.

Preparation of aqueous and plant extracts:

The leaves and fruits *Physalis angulata* were shade dried and pounded into powder using a pestle and motor. The water extracts of the medicinal plant were prepared by soaking 100 grams of the dried and pounded plant material in 1 liter of water for 72 hours and were filtered using Whitman No 1 paper. The filters were kept in the refrigerator until when needed for use.

Phytochemical screening:

The phytochemical test of the three medicinal plants was perform using the methods described by Harborne (1984), Edeoga *et al.*, (2005) and Yadav and Agarwala (2011), and Wadood *et al.*, (2013) these test was based on visual observation of color or precipitate formation after addition of a specifically plant sample were re-dissolved in 10ml of distilled

water and was subjected to qualitative phytochemical screening for saponins, tannins, alkaloids, flavonoids, phytosterols, glycosides, and terpenoids.

Cytological Analysis

To obtain root tip for cytological analysis, seeds were germinated in perforated plastic containers with very fine loam soil. To break dormancy in the seeds and allow for quick germination. Seeds were pre-soaked in 1% potassium hydroxide for 12 hours; watering was done on daily basis until germination.

Pretreatment and Fixation

When the germinating roots reached 2.5cm long, they were excised using the pointed end of a pair of forceps. This excised was done between 7.30 – 8.30 am when cells of the growing roots are known to be in active division (West and Beemster, 2004). The excised root tip was immediately dropped into a solution of 0.05% colchicine ($C_{22}H_{35}O_4$) for pretreatment. Pretreatment in colchicine was last for 5 hours, at the end of which the root tips were washed thorough in running tape water and placed in glacial acetic alcohol 1:3 v/v for 24 hours for fixation. After fixation, the root tips were hardened in absolute ethanol for one hour and washed in 30% ethanol for 3 hours before storing in 70% ethanol in the refrigerator at 4°C until required for use.

Hydrolysis, Staining, and Squashing

The root tips were picked from the 70% ethanol using a clean pair of forceps into a test tube containing distilled water. After 15 minutes of repeated changes in distilled water, the root tips were rinsed in two quick changes 1 NHCL; first in cool, followed by warm 1 NHCL. The root tip was then hydrolyzed in 1 NHCL for 8-10 minutes in a water bath regulated at 60°C (Petersen and McLaughlin, 2016). On removal from the water bath, the root tips were rinsed again in cold 1NHCL to arrest the process of hydrolysis, and again washed in distilled water for another 15 minutes. At the end of the last washing time, the root tips were sufficiently softened for squashing. Squashing was done by placing one root tip at a time on a clean dry glass slide using a pair of pointed forceps. The young meristematic portion was cut out to allow for the discarding of the older portion. One drop of 2% acetic orcein ($C_{28}H_{24}O_7N_2$) was added to the meristematic root tip and allow for 60 seconds for proper stain absorption. The stained root tip was carefully covered with a clean cover slip. Gentle taps on the coverslip using the blunt end of a mounted pin ensured proper squashing of the root tips. The whole set-up was allowed to stand for five minutes for deep staining of the chromosomes to take place. The slide was mounted and viewed, first with the X10 objective of an optical binocular research microscope, slides with good metaphase stages containing well-spread chromosomes were first drained of excess stain and then sealed using Ladies white vanish. When the seal dried, a light microscope coupled with a digital camera was used for observation, 200 cells were scored per slide, and photographs of the cell were made.

Statistical Analysis

The computer software motic images plus 2.0ml was used to capture, organize, and analyzed data from the microscope. A descriptive statistical function was employed in the preliminary analysis of data. The percentage of aberrations was analyzed using analysis of variance (ANOVA).

Result and Discussion

Phytochemical screening

The qualitative phytochemical screening of aqueous plant extracts of *physalis angulata* leaves and fruit extracts was based on visual observation of colored complex formation. The screened phytochemical classes were present in the samples as indicated in table 1 below.

Table 1: Phytochemical screening of *Physalis angulata* leaves and fruits extracts

<i>Phytochemical classes</i>	<i>Test</i>	<i>Aq. Leaves extracts</i>	<i>Aq. Fruits extracts</i>
Tannins	Ferric chloride test	+	+
Alkaloids	Mayer's test	+	+
Saponins	Froth test	+	+
Terpenoids	Salkowski test	+	+
Glycosides	Keller-Killiani test	+	+
Flavonoids	Ammonia test	+	+
Sterols	Salkowski test	+	+
Phenol		-	-

(+) = present, (-) = not present

Phytochemical Screening of *Physalis angulata* aqueous leaves and fruits extracts revealed the presence of Tannins, alkaloids, Saponins, terpenoids, glycosides, and Flavonoids, but phenol is negative as shown in (Table 1) above.

The phytochemical screening revealed the presence of some major bioactive compounds namely: Alkaloids, flavonoids, saponins, Tannins, glycosides, sterols, and terpenoids, the findings of this study agreed with the findings of Dubey (2011), and (Saboo *et al.*, 2001) regarding the presence of Tannins, Triterpenoids, and Saponins in the methanol extract of the *B. aegyptiaca* stem bark. Also, they agreed with the results of Padmapriya *et al.* (2020) regarding the presence of Saponins. However, it differs from the results of all the above three authors regarding the alkaloids which were detected by this study but not detected by them. Several previous phytochemical studies (Chand *et al.*, 2001) detected the presence of alkaloids in the stem bark but with other extracts rather than methanol. These controversial results between the different authors regarding the presence or absence of Alkaloids in the plant extracts may be attributed to the variation in the environmental factors that influence the plant growth in its different habitats.

However, several studies did detect these compounds in this part of the plant but with ethanol and aqueous extracts. This may indicate that the detected compounds may vary with the variation of the solvents. The presence of this phytochemical implies that these plants may be used for medicinal purposes. This result also is in agreement with the findings of Saxena Jyoti *et.al* (2012) isolated ten phytochemicals (Carbohydrate, Proteins, Starch, Amino acids, Steroid, Glycoside, Flavonoid, Alkaloid, Tannin, and Saponin) from Methanolic extracts of rhizomes of *Curcuma longa*.

Table 1: Chromosomal aberration observed in the root cell of *A. cepa* treated with leaf extract of *Physalis angulate*

Conc. g/ml	Total cell scored	Chromosomal aberration													% of aberration cells
		TNCD	PP	L	AB	D	VM	BN	NL	F	CG	MN	US	TNAC	
NC	200	180	0	0	0	0	0	0	0	0	0	0	0	0	0 ^a
PC	200	154	7	3	2	3	4	0	5	6	2	3	10	45	22.5 ^{bc}
5	200	176	0	0	0	9	0	0	0	0	0	0	0	0	0 ^a
10	200	171	0	2	1	2	1	0	1	0	1	1	4	13	6.5 ^{ab}
15	200	163	1	3	1	1	3	1	3	2	0	4	7	26	13 ^{bc}
20	200	150	6	4	1	0	5	3	0	6	3	6	6	41	20.5 ^{cd}

PP = Polyploidy, D= dissolution, V= vagrant metaphase, BN= Binucleate, L = Laggard, NL = Nuclear lesion, F= Fragmentation, CG= Chromosome gap, AB = Anaphase bridge, MN= Micronuclei, US= unequal separation TNCD= Total number of dividing cell, TNAC= Total number of aberrant cell.

The result on table 6 above shows that 10g is not significantly different from 5g (P =0.122) and 15g (P = 0.122), but 15g is significantly different from 5g (P =0.003), but not statistically different from 10g (P =0.122). 20g is significantly different from 5g (P =0.000), 10g (P = 0.002), but not statistically different from 15g (P = 0.097). Positive control is statistically different from 5g (P =0.000), 10g (P =0.005), 15g (P =0.026), but not statistically different from 20g (P=0.549).

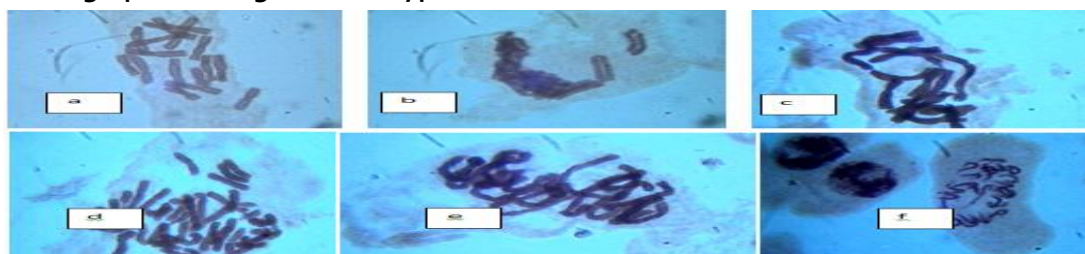
Table 2: Chromosomal aberration observed in the root cell of *a. cepa* treated with fruits extract of *physalis angucalate*

Conc. g/ml	Total cell scored	Chromosomal aberration													% of aberration cells
		TNCD	PP	L	AB	D	VM	BN	NL	F	CG	MN	US	TNAC	
NC	200	180	0	0	0	0	0	0	0	0	0	0	0	0	0 ^a
PC	200	154	7	3	2	3	4	0	5	6	2	3	10	45	22.5 ^c
5	200	170	0	0	0	9	0	0	0	0	0	0	0	0	0 ^a
10	200	168	3	2	0	5	1	3	0	3	2	0	3	22	11 ^b
15	200	150	6	6	2	4	3	2	1	2	3	0	5	34	17 ^{bc}
20	200	141	6	8	4	5	0	4	3	4	6	2	7	49	24.5 ^c

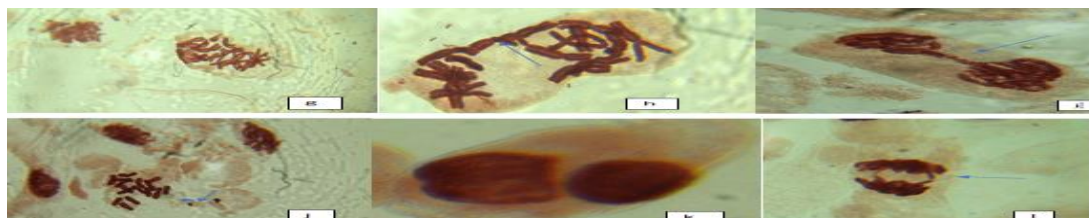
PP = Polyploidy, D= dissolution, V= vagrant metaphase, BN= Binucleate, L = Laggard, NL= Nuclear lesion, F= Fragmentation, CG= Chromosome gap, AB = Anaphase bridge, MN= Micronuclei, UE unequal separation, TNCD= Total number of dividing cells, TNAC= Total number of aberrant cell.

In table 7 above the result shows that the percentage of aberrations on the root tips of *A. cepa* treated with *P. angulate* fruits extracts at 10g is significantly different from 5g ($P = 0.012$), but not statistically different from 15g ($P = 0.161$), but root tips treated with 15g of fruits extract is significantly different from 5g ($P = 0.000$). 20g is significantly different from 5g ($P = 0.00$) and 10g ($P = 0.002$), but not statistically different from 15g ($P = 0.081$) and positive control ($P = 0.638$).

Macrograph showing different types of chromosomal aberrations



(a) normal cell with $2n=16$ (b) Stickiness (c) Chromosome gap (d) Nuclear lesion (e) Polyploidy (f) vagrant metaphase



(h) Laggard, (i) Anaphase bridge with dissolution (j) Fragmentation (k) Binucleate cells (l) Anaphase bridge

This assay demonstrates alterations in all phases of the cell cycle, which are considered evidence for mutagenic effects induced by clastogenic or anagenic agents (classified according to the type of alteration induced) (Vidakovic'-cifrek *et al.*, 2002). Some of these alterations, such as chromosomal breaks and micronuclei (MN), are chromosomal aberrations used to evaluate mutagenicity (Sobral *et al.*, 2013). The increased frequency of MN and Chromosomal aberrations in the *A. cepa* are strong evidence for the mutagenicity of the substance evaluated (Ribeiro *et al.*, 2015). It is also known that DNA damage caused by mutagenic compounds depends on the intensity, duration of exposure, and the efficiency of DNA repair activated by the exposure (Pedrazzini *et al.*, 2012).

Vidakovic-Cifrek Z, Pavlica M, Regula I, Papes D (2002) Cy-togenetic damage in shallot (*Allium cepa*) root meristems induced by oil industry "High-Density Brines". Arch Environ Contam Toxicol 43:284–291.

Some of the cellular changes found during the analysis of *Physalis angulata* plant extract were polyploidy, dissolution, Vagrant metaphase, stickiness, Binucleate, laggard, nuclear lesion, fragmentation, Chromosome gap, Anaphase bridge, Bridge, and unequal separation, (plate i-xii). It is known that anaphase bridges are formed during an unequal exchange of chromatids or by breakage and fusion of chromosomes and chromatids, and these bridges can cause structural chromosomal mutations (El-Ghamery *et al.*, 2000). Usually, chromosome bridges are found when *A. cepa* suffers from clastogenic damage. Chromosome stickiness has generally been inflicted by highly toxic agents and could lead to cell death (Barbe'rio *et al.*, 2011). MN formation is the result of chromosome breaks and lagging chromosomes. It is reported that chromosomal bridges results from the chromosome break- fusion cycle (Gomurgen 2005) and are the initiator of aneuploids and polyploids. Lagging chromosome results from spindle malfunction, which is known to be induced by heavy metals including Pb. Rucinka *et al.*, (2004) also reported that the Clastogenic effect of metals, are mostly due to the inhibition of the activity of genome-repairing enzymes leading to cellular death which cannot recover from the damage and thus increase the frequency of aberrations as a new cycle begins.

The chromosomal aberrations observed in the root tip of *Allium cepa* suggest that the medicinal plants extract *Physalis angulata* exerts mutagenic effect on the mitotic chromosome of *Allium cepa*. supporting the view that this bioassay works well for evaluating the mutagenicity of medicinal plant extracts on the root tip of *Allium cepa*. In addition to the chromosome fragments, stickiness was also observed and in general, it was possible to observe an increase of different abnormalities as the plant extracts concentration increased and a decrease in normal cells. a strong cytotoxic and mutagenic effect of plant extracts was observed, supported by the great occurrence of sticky metaphases, leading to cellular death. This is in agreement with Fernandes *et al.*, (2007), which reported that the cytotoxicity levels of an agent can be determined by increases or decreases in the percentage of aberrations. Therefore, with the progressive increase in the number of aberrant cells as the concentrations increase significantly compare to negative control indicates inhibition in cell proliferation (by the action of plant extracts or chemical substance) (Fernandes *et al.*, 2007).

References

- Akinboro, A., Mohammed, K., Selestin, R., & Muniandy, V. R. (2011). Genotoxicity Assessment of Water Samples from the Sungai Dua River in Pulau Pinang, Malaysia, Using the *Allium cepa* Test. *Tropical Life Sciences Research*, 22(2), 23-35.
- Amoah P, Drechsel P, Abaidoo RC, Ntow WJ. (2006) Pesticide and pathogen contamination of vegetables in Ghana's urban markets. *Arch Environ Contam Toxicol*. Jan;50(1):1-6
- Anghili, K., Makhzan, M.H. A. advieh, T. (2001). The traditional usage of golden berry Rah-e-Kamal Press, 2001.
- Bianchi, Clara; Mendoza, Luciano Pedro Oscar; Fernández, Laura; Natali, María Paula; Meza, Amalia; Moirano, Juan (2016). Time series of atmospheric water vapour and troposphere zenith total delay,

- over Central and South America, from a homogeneous GNSS reprocessing (MAGGIA ZTD & IWV Solution 1). PANGAEA. <https://doi.pangaea.de/10.1594/PANGAEA.858234>
- Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV, Schiff S, Ślusarczyk J, de Souza CP, Srivastava A, Sutan A, Papini A (2018). An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. *Caryologia* 71(3):191-209.
- Bourgaud, Frederic & Gravot, Antoine & Milesi, Sandrine & Gontier, Eric. (2001). Production of plant secondary metabolites: A historical perspective. *Plant Science*. 161. 839-851.
- Camparoto ML, Teixeira RO, Mantovani MS, Vicentini VEP (2002). Effects of *Maytenus ilicifolia* Mart. and *Bauhinia candicans* Benth infusions on onion root-tip and rat bone-marrow cells. *Genetics and Molecular Biology* 25(1):85-89
- Chand S, Anwar M, Patra DD. Influence of combined application of farm yard manure (FYM) and inorganic fertilizers on herb, essential oil yield and nutrients accumulation in menthol mint (*Mentha arvensis*). *J of Medi and Arom Plant Sci*. 2001; 23(2):29-33.
- deOliveira, A. M. L. Malunga, N. Camila Perussello, A. Rosemary Trust Beta, Ribani, Hoffmann (2020). Phenolic acids from fruits of *Physalis angulata* L. in two stages of maturation. *South African Journal of Botany*, Volume 131, 2020, Pages 448-453,
- Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigeria medicinal plants. *African Journal of Biotechnology*. 4:685-688.
- El-Ghamery AA, El-Nahas AI, MansourMM (2000) The action of atrazine herbicide as an indicator of cell division on chromosomes and nucleic acid content in root meristems of *Allium cepa* and *Vicia faba*. *Cytology* 65:277-287.
- Fachinetto JM, Bagatini MD, Silva ACF, Tedesco SB (2007). Efeito antiproliferativo das infusões de *Achyrocline satureioides* DC (Asteraceae) sobre o ciclo celular de *Allium cepa*. *Revista Brasileira de Farmacognosia* 17(1):49-54.
- Fachinetto JM, Tedesco SB (2009). Atividade antiproliferativa emutagênica dos extratos aquosos de *Baccharis trimera* (Less.) A. P. de Candolle e *Baccharis articulate* (Lam.) Pers. (Asteraceae) sobre o sistema teste de *Allium cepa*. *Revista Brasileira de Plantas Medicinai*s 11(4):360-367
- Fiskesjö G. (1985). The *Allium* test as a standard in environmental monitoring. *Hereditas*. 1985;102(1):99-112.
- FISKESJÖ, G. (1985). The *Allium* test as a standard in environmental monitoring. <https://onlinelibrary.wiley.com/action/doSearch?ContribAuthorRaw=FISKESJ%C3%96%2C+GEIRID>
- Harborne, J.B. (1998). *Phytochemical method: A guide to modern technique of plant analysis*. Edu 2, Chapman and Hall Ltd., London. P.282.
- Hussein, Rehab & El-Anssary, Amira. (2019). *Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants*. ISSN 0254-6299,
- Jayaraj, P. (2010). Regulation of traditional and complementary medicinal products in *Malaysia*. *Int Journal Green Pharm* 4(1), 10-14. <http://dx.doi.org/10.4103/0973-8258.62158>
- Knoll MF, Silva ACF, Tedesco SB, Canto-Dorow TS (2006). Effects of *Pterocaulon polystachyum* DC. (Asteraceae) on onion (*Allium cepa*) root-tip cells. *Genetics and Molecular Biology* 29(3):539-542
- Krishnaraju, A. V., Rao, T. V. N., Sundararaju, D., Vanisree, M., Tsay, H.-S., & Subbaraju, G. V. (2006). Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using Artemiasalina (Brine Shrimp Test). *International Journal of Applied Science and Engineering*, 4(2), 115-125.
- Leme, D.M., Marin-Morales, M.A., (2009). *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Research/Reviews in Mutation Research* 682,71-81
- Liman R, Başbuğ B, Ali MM, Acikbas Y, Ciğerci İH (2021). Cytotoxic and genotoxic assessment
- Liman R, Taşdemir B, Gökçe S, Amaç E, Ciğerci İH, Korcan SE (2020). Cytogenotoxic and forced

- Lubini G, Fachinetto J, Laughinghouse H, Paranhos J, Silva A, Tedesco S (2008). Extracts affecting mitotic division in root-tip meristematic cells. *Biologia* 63(5):647-651.
- Luximon-Ramma, A., Bahorun, T., & Crozier, A. (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. *Journal of the Science of Food and Agriculture*, 83(5), 496-502.
- Macar O (2020). Multiple toxic effects of tetraconazole in *Allium cepa* L. meristematic cells. Environ
- Maity S, Chatterjee A, Guchhait R, De S, Pramanick K (2020). Cytogenotoxic potential of a hazardous
- Mazzio, E. A., & Soliman, K. F. (2009). In vitro screening for the tumoricidal properties of international medicinal herbs. *Phytotherapy research: PTR*, 23(3), 385–398.
- Padmapriya, K & Barathi, S & Munisamy, Pandeewaran. (2020). Phytochemical Screening Test for Eleven Different Medicinal Plants in and Around Dindigul City.
- Pedrazzani R, Ceretti E, Zerbini I, Casale R, Gozio E, Bertanza G, Gelatti U, Donato F, Feretti D (2012) Biodegradability, toxicity and mutagenicity of detergents: integrated experimental evaluations. *Ecotoxicol Environ Saf* 84: 274–281.
- Petersen, J. McLaughlin, S. (2016). Laboratory Exercises in Microbiology: Discovering the Unseen World Through Hands-On Investigation. City University of New York (CUNY) CUNY Academic Works Open Educational Resources. Queensborough Community College
- Praveen, P. Reddy, Reddy, G.S.N. and Sulochana, M.B. (2010). Screening of β -Fructofuranosidase Producers with High Transfructosylation Activity and its 32 Experimental Run Studies on Reaction Rate of Enzyme. *Journal of Biological Sciences*, 10: 237-241.
- Puente, L.A., Pinto-Munoz, C.A., Castro, E.S., Cortes, M. (2011). *Physalis peruviana* Linnaeus, the multiple properties of highly functional fruit: A reviewing. *Food Res Int*. 2011; 44:1733-1740.
- Ribeiro JC, Mota-Pereira J, Silverio J, Carvalho S, Fonte D, Ramos J. Moderate exercise improves depression parameters in treatment-resistant patients with major depressive disorder. *J Psychiatr Res*.
- Ribeiro, D. L., Silveira, M. A., Dos Santos, T. A., Vieira, G. M., Cechinato, C. N., Kazanovski, M., & Grégio d'Arce, L. P. (2016). Mutagenicity of two herbicides widely used on soybean crops by the *Allium cepa* test. *Cytotechnology*, 68(4), 1215–1222.
- Rosculete C, Bonciu E, Rosculete E, Olaru L (2019). Determination of the environmental pollution
- Saxena, P.N., Gupta, S.K., Murthy, R.C., (2012). Carbofuran induced cytogenetic effects in root meristem cells of *Allium cepa* and *Allium sativum*: A spectroscopic approach for chromosome damage. *Pesticide Biochemistry and Physiology* 96, 93-100.
- Sobral O, Marin-Morales MA, Ribeiro R (2013) Could contaminant induced mutations lead to a genetic diversity overestimation? *Ecotoxicol*. doi:10.1007/s10646-013- 1079-4.
- Srivastava, A. K. Singh D (2020). Assessment of malathion toxicity on cytophysiological activity, DNA
- Stanley Wilson, M. & O.A.W. Handy, (2022). Afropolitan Journals Investigation of Phytochemical and Trace Metal Elements in Medicinal Plants Obtained in Girei Local Government Area, Adamawa State, Nigeria. *African Journal of Advanced Sciences & Technology Research*, 8(1), 32–47.
- Tariq, A.L. and Reyaz, A.L. (2013). Significance and importance of the phytochemicals present in *Terminalia chebula*. *International Journal of Drug Development and Research*. 5(3):256-262.
- Tchacondo, T., Korou, T., Batawila, D.S., Agban, K., Ouro-Bang'na, A., Anani, K., Gbeassor, K.T., and Desouza, M.C. (2011). Herbal remedies and their adverse effects in Tem Tribe Traditional Medicine in Togo. *African Journal of Traditional and Complementary Alternative Medicine*. 8(1):45-60.
- Tedesco SB, Laughinghouse IV HD (2012). Bioindicator of Genotoxicity: The *Allium cepa* Test. In: Srivastava JK (ed.) Environmental Contamination. Rijeka Croatia: InTech. pp. 137-156.

- Vidakovic-Cifrek Z, Pavlica M, Regula I, Papes D (2002) Cytogenetic damage in shallot (*Allium cepa*) root meristems induced by oil industry "High-Density Brines". *Arch Environ Contam Toxicol* 43:284–291.
- West, G., Inzé, D., & Beemster, G. T. (2004). Cell cycle modulation in the response of the primary root of *Arabidopsis* to salt stress. *Plant physiology*, 135(2), 1050–1058.
- Zhang, H, Abbas, K. S. Robert, J. G. Juan JA, Xiaoqin T, Victor WD, Mark SC, Kelly K, Rao G, Barbara NT, (2011), Cytotoxic oleanane-type saponins from *Albizia inundata*, *Journal of Nat. Prod.*,74, 2532–2544.