Journal of Applied Life Sciences International

24(6): 25-35, 2021; Article no.JALSI.75867
ISSN: 2394-1103

Evaluation of Cytotoxic Effects of Methanolic Extract and Fractions of *Mirabilis jalapa* (L.) Leaf

Sekinat Okikiola Azeez[1*], Aanuoluwapo Nifemi Olowu[1], Anyim Godwin[2], Joseph Tosin Apata[2] and Bolajoko Ayinke Akinpelu[2]

[1]Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.
[2]Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. The work was designed by authors SOA and BAA. The cytological aspect was done by authors SOA, ANO, AG and BAA did the extraction, Allium cepa bioassay and phytochemical screening. Authors BAA and JTA carried out the Brine Shrimp bioassay. All the authors read and approved the final manuscript.

.

Article Information

DOI: 10.9734/JALSI/2021/v24i630242
Editor(s):
(1) Dr. Palanisamy Arulselvan, Universiti Putra Malaysia, Malaysia.
Reviewers:
(1) Rajesh A. Shastri, SETs College of Pharmacy, India.
(2) Ravi Manne, USA.
Complete Peer review History: https://www.sdiarticle4.com/review-history/75867

Original Research Article

Received 11 August 2021
Accepted 23 October 2021
Published 29 October 2021

ABSTRACT

This study examined the potential cytotoxicity of *Mirabilis jalapa* L. methanolic crude leaf extract and its fractions against brine shrimp nauplii (*Artemia salina* L.) and *Allium cepa* L. roots. The leaf extraction was done according to standard technique and crude extract was partitioned using n-hexane, water, ethyl acetate and butanol to obtain their respective fractions. *Allium cepa* root growth inhibition of *M. jalapa* methanolic crude extract and fractions were evaluated as well as brine shrimp lethality of the fractions based on standard methods. Also, phytochemical screening of the methanolic crude leaf extract was carried out according to standard methods. The result showed that *M. jalapa* methanolic crude leaf extract caused a significant reduction in cell mitotic index (32.96%) compared with the control (52.13%). The butanol fraction produced the highest mitotic inhibitory activity on *A. cepa* cell division at 0.3 mg/ml. Moreover, the butanol fraction produced the highest percentage lethality (LC_{50} 1.45 µg/ml) against brine shrimp nauplii. There was a strong correlation between brine shrimp lethality and mitotic cell inhibition with butanol fraction as the most potent in both models. The methanolic leaf crude extract tested positive for alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tanins and triterpenes. The methanolic crude extract of

**Corresponding author: E-mail: sekinatokiki@gmail.com, azeezs@oauife.edu.ng*

M. jalapa leaf and its fractions exhibited effective cytotoxic effect on *A. cepa* and brine shrimps. Butanol fraction, with the most cytotoxic activity among the tested extracts, demonstrates a promising source for novel anticancer agents.

Keywords: Mirabilis jalapa; cytotoxicity; brine shrimps; Allium cepa; mitotic index.

1. INTRODUCTION

Mirabilis jalapa L. belongs to a small family, Nyctaginaceae consisting majorly New world tropical and subtropical species [1]. It is a perennial weedy plant found in disturbed areas, meadows and fields. It possesses different flower colors which include yellow, white, magenta and pink [2, 3]. *Mirabilis jalapa* is a well-known ornamental plant that is cultivated throughout the world for its beautiful and pleasing fragrance flowers [2,3]. It is popularly known as 4 0' clock plant and it is called "Tonaposo" among Yoruba people of Nigeria [4]. The leaves are ovate shape, green, and bitter with a characteristic odour [5,6]. A chromosome number of 2n = 54 [7] and 2n= 58[8] was reported in *M. jalapa*.

Different parts of *M. jalapa* have been studied by many researchers. Phytochemical constituents such as phenols, alkaloids, glycosides, flavonoids, saponins, steroids, tanins, triterpenes and anthraquinones were reported to be present in different parts of *M. jalapa* [6]. A wide range of biological activities of the plant extracts has also been documented. These include anti-inflammatory, anti-bacterial, anti-microbial, anti-oxidant**,** anti-cancer, anti-nociceptive, anti-spasmolytic, anti-viral, anti-fungal, anti-diabetic, anti-helmintic, anti-malarial anti-stress and immune stimulating activities [3, 5, 9, 10].

Mirabilis jalapa is used in the treatment of various ailments such as dropsy (inflammatory oedema), fever, menstrual disorders, constipation, and diarrhea. In addition, it is used as aphrodisiac, diuretic and laxative (cleanser) [2,3]. In Nigeria, the leaves are consumed as memory enhancer [4]. Moreover, an edible red dye for coloring food was obtained from *M. jalapa* flower [2]. Its leaves have also been reported to be cooked as food during an emergency case only [3]. In spite of these acclaimed uses in ethno medicine, the whole plant of *M. jalapa* was reported to be poisonous [11]. Hence, this study was undertaken to investigate the potential toxic effects of *M. jalapa* using *Allium cepa* and Brine Shrimp models.

Allium cepa assay is an important *in vivo* test used by researchers to evaluate the toxicity of any chemical agent or substance. The roots are grown in direct contact with the test material enabling possible damage to DNA of humans to be predicted [12,13,14]. Additionally, brine shrimp lethality assay (BSLA) is a simple and economical bioassay used globally for testing the efficacy of chemical agents and plant extracts for presence of toxic principle(s) or effects of test sample on acute overdose [15,16]. Such lethality in a simple organism like *Artemia salina.* (Brine shrimp nauplii) has been employed by various investigators as suitable tool for screening chemical agents for toxic effects. In BSLA, the organism is directly exposed to the test sample, and after 24 hours, the number of nauplii that survive is counted and the percentage mortality is determined using an appropriate equation [14, 17].

2. MATERIALS AND METHODS

2.1 Plant Sample Collection

Fresh leaf sample of *Mirabilis jalapa* was collected from Ido Osun, Ile-Ife, Osun State, Nigeria (7°27" 7.49N, 4° 34" 4.56E) and authenticated at the IFE Herbarium, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. A voucher specimen with voucher number IFE-17999 was deposited at IFE Herbarium (Fig 1).

2.2 Preparation of *M. jalapa* Leaf Extract

The leaves were air dried for three weeks and milled into powder. The powdered sample (15 g) was extracted with 80% (v/v) methanol for 48 h, and filtered with No 1 Whatman filter paper. The residue was soaked in freshly prepared 80% (v/v) methanol and the process was repeated till a clear solution was obtained. The filtrates were pooled and concentrated *in vacuo* on a rotary evaporator at 40 °C to yield the methanolic crude extract.

2.3 Partitioning of Crude Methanolic Extract of *M. jalapa* Leaf

The crude methanolic leaf extract of *M. jalapa* was dissolved in distilled H_2O and successively

partitioned with n-hexane (n-Hex), ethyl acetate (EtOAc), butanol (BuOH) and water (Aqeous) [13]. The solvent fractions were concentrated *in vacuo* on rotary evaporator to produce n-hexane, ethyl acetate, butanol and aqueous fractions respectively.

2.4 *Allium cepa* Bioassay

Methanolic crude extract and fractions (n-hexane, ethyl acetate, butanol and aqueous) of *M. jalapa* leaf were evaluated for root growth inhibitory potential using *Allium cepa* model [13]. Fresh onion bulbs were purchased from Ile-Ife local market and sundried for three weeks. The rooting was initiated in distilled water for 48 hours. The sprouted bulbs were planted separately in 0.0, 0.10, 0.20, 0.30 and 0.40 mg/ml of the extract and fractions (n-hexane, ethyl acetate, butanol and aqueous) of *M. jalapa* for 24 hours while the sprouted bulbs in distilled water served as the control. Thereafter, the roots were harvested and fixed in acetic acid/ethanol (1:3 v/v) for 24 hours and stored in refrigerator for further analysis. Five roots from each bulb were measured and the mean root length was calculated.

2.5 Cytological Examination of the *Allium cepa* Root Tips

The fixed roots were hydrolyzed in HCl (18% v/v) for 10 minutes and the root tips were squashed and stained with FLP-orcein for 15 minutes [14]. A total of twenty-five fields was viewed at random from five slides; the total number of cells undergoing mitotic division and the total number of non-dividing cells were counted. Photomicrographs of the cells were taken using ACU-scope trinocular microscope (ACCU-scope 33001 LED trinocular microscope fitted with 3.2 MP (MOS digital camera). The mitotic index was calculated as:

Mitotic Index = (Number of dividing cells ÷ Total number of cells) × 100

2.6 Brine Shrimp Lethality Bioassay

The various fractions of *M. jalapa* were assessed for lethality to brine shrimp larvae following standard method [17]. The eggs of brine shrimp were hatched using brine shrimp eggs in a hatching chamber (1L) filled with sterile artificial seawater under constant aeration for 48 hours. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay.

Each fraction was screened separately at 10, 100 and1000 µg/ml in 10 ml sea water solutions with 1% DMSO (*v/v*) as vehicle that dissolved the test sample. With the aid of a Pasteur pipette, 10 living nauplii were transferred to each test concentration, 1% DMSO or sea water (negative control) as well as Diclofenac solution (positive control) in vials maintained at room temperature for 24 hours under the light.. After 24 hours, the number of nauplii survived in each vial was counted with the help of magnifying glass. The assay was performed in triplicate. The lethal concentration that produced 50% mortality after 24 hours of exposure (LC_{50}) was determined using the probit method. The LC_{50} values greater than 1000 µg/ml for plant extracts were considered inactive [17].

2.7 Phytochemical Screening of *M. jalapa* Methanolic Crude Leaf Extract

The phytochemical screening of the methanolic leaf crude extract of *M. jalapa* was carried out according to standard methods [18,19].

2.8 Statistical Analysis

Data obtained were subjected to statistical analysis using One-Way Analysis of Variance. Data was considered significant at $p \leq 0.05$. Data were expressed as mean with standard error in Brine Shrimp assay. Duncan's multiple range tests was employed to determine the significant difference among the mitotic parameters for different concentrations in *A. cepa* assay.

3. RESULTS AND DISCUSSION

3.1 Effect of Methanolic Crude Leaf Extract of *M. jalapa* on *Allium cepa* Roots and Mitotic Index

At higher concentrations of the plant extract, the *Allium cepa* roots showed little or no further growth after 24 hours of exposure. Roots placed in control (distilled water) and 0.1 mg/ml appeared healthier, longer and bigger compared to the roots exposed to higher concentrations. The highest root growth inhibition (38.09%) was recorded at 0.2 mg/ml; while the least inhibition (13.80%) was obtained at 0.1 mg/ml. Also, there was a significant reduction in the mitotic index (32.96%) of the roots treated with different concentrations of *M. jalapa* when compared with the control (52.13%). The effects of the methanolic crude extract on *A. cepa* root growth

and mitotic index were not dose dependent and no chromosomal aberration was observed (Table 1; Fig 2).

Toxic effects of pollutants, drugs or herbal preparations have been investigated by analyzing macroscopic parameters (such as root number and root growth) while genotoxicity and cytotoxicity were evaluated by investigating the cytological parameters [20]. Root growth inhibition over 45% indicates presence of toxicants with sub-lethal effects on the test plants [21]. However, the highest root growth inhibition recorded in the methanolic crude extract was 38.09% which showed that the tested crude extract of *M. jalapa* did not possess any toxicant with sub-lethal effect. The percentage root growth inhibition was observed to be correlated with the mitotic activity of the cells. Nevertheless, the observed wilted and stunted growth in the immersed roots in the methanolic crude extract and fractions of *M. jalapa* at concentrations higher than 0.1 mg/ml were indication of cytotoxicity. Stunting and wilting roots are considered as signs for anti-mitotic activities [22]. Significant decrease in mitotic index suggests mito-suppressive action of the compound, indicating that *M .jalapa* extract interfered with the normal sequence of cell cycle which consequently reduced the number of dividing cells [23].

Fig. 1. Habit of *Mirabilis jalapa*

Table 1. Effect of the *M. jalapa* methanolic leaf crude extract on root growth and mitotic index

Conc. (mg/ml)	Root length Mean (cm)	Growth inhibition (%)	Mitotic Index (%)
0	2.10±0.16^{a}	0.00	52.13^{a}
0.1	1.81±0.24^{b}	13.80^{d}	42.73^{b}
0.2	1.30±0.14^{e}	38.09^{a}	32.96^{d}
0.3	1.57±0.21^{d}	25.24^{b}	38.37^{e}
0.4	1.78±0.21^{c}	15.23^{c}	40.64^{c}

Values with different letters are significantly different from each other at p≤0.05

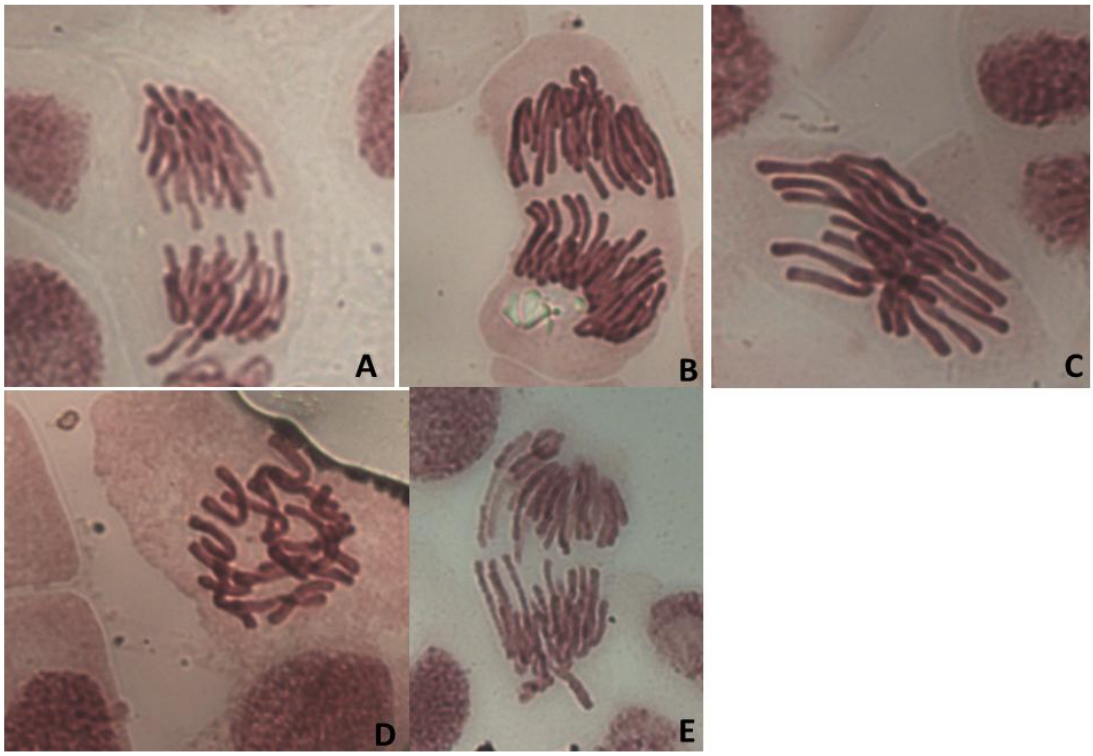

Fig. 2.Stages of Cell division in *A. cepa* at different concentrations
A. Anaphase (Control); B. Anaphase (0.1mg/ml); C. Metaphase (0.2mg/ml); D. Metaphase (0.3mg/ml); E. Anaphase (0.4mg/ml)

3.2 Effect of *M. jalapa* Leaf Fractions on *Allium cepa* Root Growth and Mitotic Index

A non-significant decrease in root length was observed at different concentrations of *M. jalapa* fractions (Table 2) except for aqueous fraction which enhanced root growth at 0.2 and 0.3 mg/ml. The butanol leaf fraction significantly inhibited the *A. cepa* root growth across all the concentrations tested. The n-hexane, EtOAc and BuOH fractions inhibited the *Allium cepa* root growth only at lower concentrations. This suggested that the butanol fraction contained significant amounts of mito-suppressive constituents which interfered with *Allium cepa* cell division. The reduction in mitotic activity could also be due to inhibition of DNA synthesis which is one of the major prerequisites for cell division [24]. In biological system, there are some surveillance mechanisms that have been in place to detect different DNA damage which act in many ways to maintain genome integrity; one of the ways is the blockage of DNA replication to slow or stop cell cycle progression [25]. Consequently, there will be reduction in cell mitotic activity. In addition, DNA breakage at the replication site is being stimulated by inhibition of DNA replication resulting in cytotoxicity [26].

Table 2. Effect of the leaf fractions of *M. jalapa* leaf extract on root length mean of *A. cepa*

Sample (mg/ml)	0.1	0.2	0.3	0.4
Root length Mean (cm)				
Control	3.54±0.17^{a}			
n-Hex Fraction	3.30±0.15^{a}	3.28±0.16^{a}	2.32±0.25^{a}	3.56±0.25^{a}
EtOAc Fraction	3.20±0.28^{a}	3.38±0.44^{a}	3.72±0.27^{a}	3.92±0.26^{a}
BuOH Fraction	1.78±0.14^{b}	2.58±0.23^{b}	2.88±0.33^{b}	3.32±0.18^{a}
Aq Fraction	2.50±0.14^{b}	4.2±0.29^{a}	4.34±0.61^{a}	3.08±0.24^{a}

Values with different letters are significantly different from each other at p≤0.05

More so, the decrease in the mitotic index might be caused by the decreasing ATP level and pressure from the functioning of the energy production center [27]. Furthermore, inhibition of DNA polymerase, which is necessary for the synthesis of DNA precursors as well as other enzymes more directly involved with spindle production, assembly or orientation, could also account for the reported antimitotic effects [28]. The BuOH leaf fraction demonstrated the highest level of potency (Table 3), followed by aqueous fraction while ethyl acetate fraction showed the least potency at inhibiting *A. cepa* cell division. The ethyl acetate fraction stimulated cell mitosis at 0.2 mg/ml but strongly inhibited cell division at higher concentrations (Table 3) and caused chromosomal aberrations (clumped metaphase), cell elongation and cell degeneration (Fig. 3). At higher concentration in n-hexane fraction, cell degeneration was observed in addition to normal cells. The absence of chromosomal aberrations in the cells treated with the methanolic crude extract indicated that the extract elicits no genotoxic effect at the concentration tested. It should be noted that crude therapeutic products are less toxic which was said to be due to synergistic interaction of the active ingredients in them [29]. However, cell and chromosomal abnormalities were observed in some cells treated with the fractions of the leaf extracts (BuOH and EtOAc). In this current study, *M. jalapa* leaf extract fractions showed both cytotoxic and genotoxic effects; indicating the toxicity of the plant extract. The cytotoxic and genotoxic activities of a plant extract are very important in evaluating cytotoxicity and screening of a prosperous pharmacological important drug [29,30].

3.3 Effect of *M. jalapa* Leaf Fractions on Brine Shrimp Lethality

The effect of the leaf fractions of *M. jalapa* on shrimp nauplii (Table 4) was concentration dependent. The highest percentage mortality was recorded after 24 hours at 1000 µg/ml. All the shrimp nauplii survived in the control medium (sea water). The butanol fraction produced the highest percentage lethality on brine shrimp nauplii (LC_{50} 1.45 µg/ml) and was considered the most potent fraction. This was followed by the ethyl acetate fraction (LC_{50} 48.33 µg/ml) while n-hexane fraction as well as aqueous fraction showed low level of potency and were considered inactive. There was a strong correlation between brine shrimp lethality and mitotic cell inhibition with butanol fraction as the most potent in both models.

In brine shrimp assay, LC_{50}<1.0 µg/ml is considered highly toxic; LC_{50} 1.0-10.0 µg/ml is toxic; LC_{50} 10 –30 µg/ml is moderately toxic; LC_{50} 30 – 100 µg/ml is mildly toxic, and > 100µg/ml is non-toxic [31]. Therefore, n-hexane and aqueous fractions, DMSO and diclofenac which had LC_{50} above 100 µg/ml were practically non-toxic. Nevertheless, butanol fraction with LC_{50} value of 1.45 µg/ml was toxic while ethyl acetate fraction (LC_{50} - 48.33 µg/ml) was mildly toxic. Plant extracts with LC_{50}< 20 µg/ml have a tendency to yield anticancer compounds [31]. Moreover, The brine shrimp lethality bioassay has been shown to have a positive correlation with cytotoxic activity in some human solid tumors which has given way to the discovery of novel active antitumoral agents [32]. Therefore, the butanol leaf fraction of *M. jalapa* has the potential to offer new lead molecules for cancer therapy. The LC_{50} cutoff point has also been extensively suggested elsewhere [32, 33]. An anti-cancer compound Englerin A was isolated from the extract of *Phyllanthus engleri* which was shown to have selective anti-cancer property against kidney cancer cells [33]. Such findings provide further corroborative evidence on the potential of brine shrimp test to predict the presence of anti-cancer compounds in plant extracts. Cytotoxic action of a drug was said to have a relationship with the disturbance of important mechanisms that are associated with cell growth, mitotic activity, differentiation and function [34] as observed in this study. Therefore, the results from this present study, suggested the presence of anticancer bioactive components in the butanol fraction of *M. jalapa* leaf.

3.4 Phytochemical Constituents

The methanolic crude leaf extract of *M. jalapa* tested positive for alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and triterpenes (Table 5). Qualitative analysis of the result showed that cardiac glycosides, steroids and triterpenes were more abundant than the rest of phytochemicals screened. The presence of alkaloids, glycosides, flavonoids, tannins, steroids and triterpenes in the plant studied correlated with other reports.[6,9,10] Phytochemicals are biologically active, naturally occurring chemical compounds found in plants. Phytochemicals have been reported to be responsible for various plant biological activities and provide health benefits for humans as

medicinal ingredients and nutrients [35,36]. Recently, attention is being given to plant secondary metabolites over the synthetic chemicals because these plant products have been shown to be useful pharmaceutically [37]. More so, the antibacterial and antioxidant activities of *M. jalapa* have been attributed to the presence of phytochemicals in the plant [38].

Table 3. Effect of the methanolic leaf fractions of *M. jalapa* on mitotic index of *A. cepa*

Conc. (mg/ml)	Control	EtOAc Fraction	n-hexane fraction	BuOH fraction	Aqueous fraction
	37.59				
0.1		33.61^{b}	21.03^{a}	2.70^{a}	21.32^{a}
0.2		43.06^{a}	25.19^{a}	0.96^{b}	10.11^{b}
0.3		30.35^{b}	17.59^{b}	0.57^{b}	4.19^{c}
0.4		11.48^{c}	21.00^{a}	1.45^{a}	7.80^{c}

Values with different letters are significantly different from each other at p≤0.05

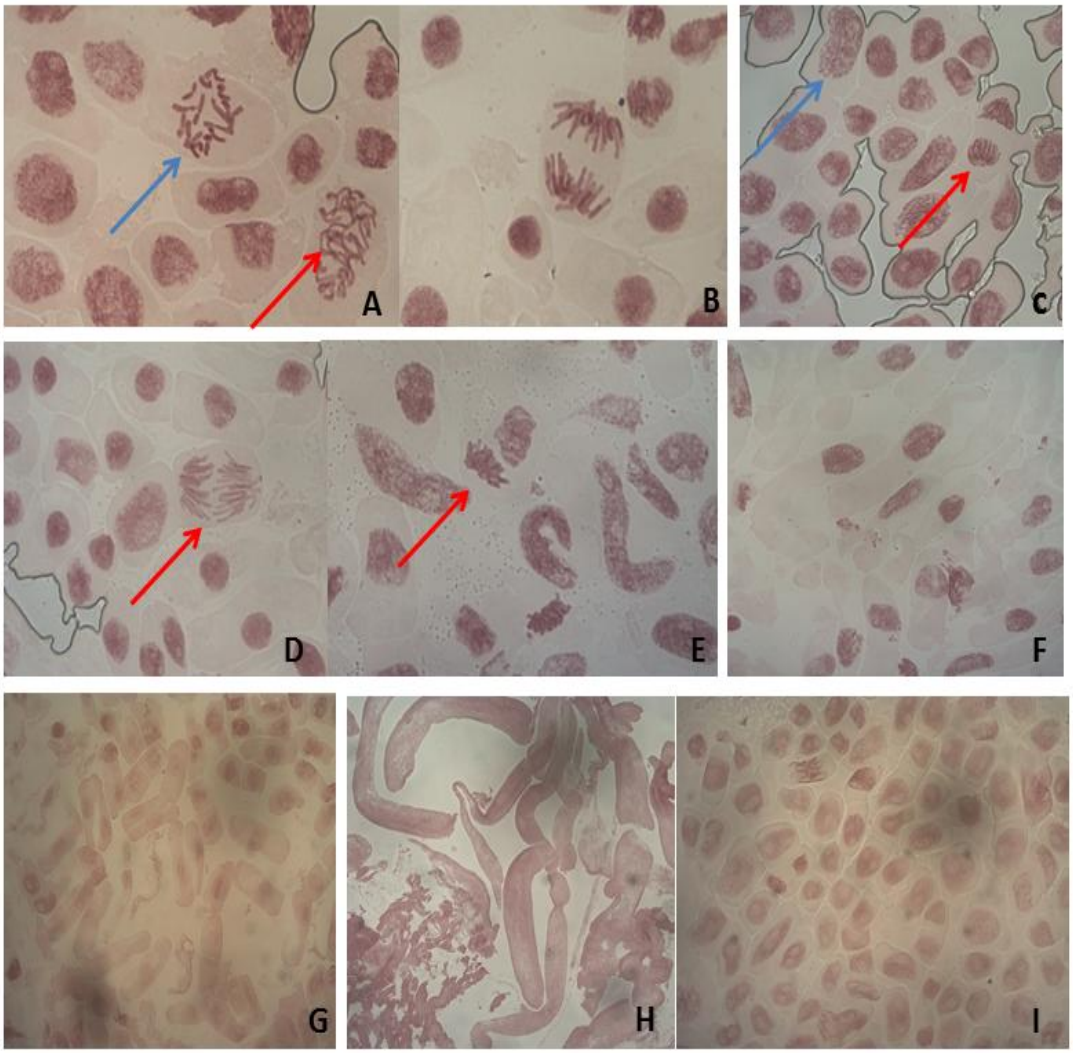

Fig. 3. Stages of cell division in *A. cepa* at different concentrations

A. Metaphase (blue arrow) and Prophase (red arrow) (DMSO); B. Anaphase (DMSO); C. Prophase (blue arrow) and Early Telophase (red arrow) (EtOAc 0.1 mg/ml); D. Anaphase (EtOAc 0.1 mg/ml); E. Clumped Metaphase (EtOAc 0.2 mg/ml); F. Elongated cells (EtOAc 0.2 mg/ml); G. Degenerated cells (EtOAc 0.4 mg/ml); H. Elongated cells (BuOH 0.1 mg/ml);I. Different stages of cell division (N-Hex 0.4 mg/ml)

Table 4. Percentage mortality of shrimp nauplii Treated with M. jalapa leaf fractions

		At 0 hour	After 6 hours	After 12 hours	After 18 hours	After 24 hours	
Test Sample	**CONC. (µg/ml)**	**Mortality (%)**	**Mortality (%)**	**Mortality (%)**	**Mortality (%)**	**Mortality (%)**	**LC_{50} (µg/ml)**
BuOH	10	0	5	20	45	85	1.45
Fraction	100	0	10	25	55	95	
	1000	0	5	30	65	95	
EtOAc	10	0	0	15	30	70	48.33
Fraction	100	0	20	25	40	75	
	1000	0	20	35	65	100	
Aqeous	10	0	0	10	40	65	3386.28
Fraction	100	0	0	15	50	65	
	1000	0	10	20	60	75	
n-Hex	10	0	0	15	35	65	1172.85
Fraction	100	0	0	25	45	75	
	1000	0	5	20	70	95	
DMSO	10	0	0	0	5	15	2648.13
(vehicle)	100	0	0	0	10	30	
	1000	0	5	0	15	50	
Diclofenac	10	0	10	5	20	85	1002.08
(control drug)	100	0	0	35	65	85	
	1000	0	35	40	80	100	
Sea water	10	0	0	0	0	0	0
	100	0	0	0	0	0	
	1000	0	0	0	0	0	

Table 5. Phytochemical constituents of *M. jalapa* crude extract

Phytochemical Constituents	**Presence**
Alkaloids	+
Cardiac Glycosides	++
Flavonoids	+
Saponins	+
Steroids	++
Tannins	+
Triterpenes	++

+ present; ++ moderately present

4. CONCLUSION

Mirabilis jalapa leaf extract exhibits cytotoxic, genotoxic and lethal effects on *A. cepa* and brine shrimps nauplii. However, the butanol leaf fraction LC_{50} value suggested that *M. jalapa* leaf is a promising source for novel anticancer agents. Therefore, further studies on isolation of potential antitumour and cytotoxic agents from the plant are strongly recommended.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Levin RA. Phylogenetic relationships within Nyctaginaceae, tribe Nyctagineae: Evidence from nuclear and chloroplast genomes. Systematics Botany. 2000;25(4):737-750
2. Mahr, S. Four o'Clock, *Mirabilis jalapa;* 2013. Available:https://wimastergardener,org/article/four-clocks mirabilis-jalapa accessed 02/10/2021
3. Rozina R. Pharmacological and biological activities of *Mirabilis jalapa* L. *Int. J. Pharmc. Res.* 2016;6(5):160-168
4. Elufioye TO, Oladele AT, Cyril-Olutayo CM, Agbedahunsi JM, Adesanya SA. Ethanomedicinal study and screening of plants used foe memory enhancement and antiaging in Sagamu, Nigeria. European J. Med. Plants. 2012;2(3):262-275
5. Ramesh BN, Mahalakshimi AM. An ethnopharmacological: Review of Four O'clock Flower Plant (*Mirabilis jalapa* Linn). J. Biol. Sci. Opin. 2014;2(6):344-348.
6. Hanani E, Prastiwi R, Karlina L. Indonesian *Mirabilis jalapa* Linn: A pharmacognostical and preliminary phytochemical investigation. Pharmacogn J. 2017;9(5) :683-688. DOI: 10.5530/pj.2017.5.108
7. Bala S, Gupta RC, Attir PB. Cytological studies on the Monochlamydeae from North India. Chromosome Botany. 2011;6:25-51
8. Verma AK, Mishra M, Singh H, Bharati, KA. Database of chromosome count of some Indian plants. Chromosome Botany. 2018;13(1):37-60
9. Sathish K, Eram, F. *Mirabilis jalapa*: Phytochemical screening and anti-stress activities of methanolic leaf extract. J. Pharmacogno. Phytochem. 2017;6(6):1502-1508.
10. Al-Snafi AE, Talab TA, Jabbar WM, Al-qahtani AM. Chemical constituents and pharmacological activities of *Mirabilis jalaba*-A review. Int. J. Pharm. Biol. Arch. 2021;1(2):34-45. DOI:https//doi.org/10.30574/ijbpsa.2021.1.20303
11. Kaplan M. Plant Listings. In: Harmful and edible plants section. 2002. Available:www.anapsid.org accessed 20/06/2019
12. Nefic H, Musanovic J, Metovic A, Kurteshi K. Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. induced by Alprazolam. Medical Arch. 2013;67(6):385-392
13. Akinpelu BA, Godwin A, Aderogba MA, Makinde AM, Azeez SO, Oziegbe M. Evaluation of anti-inflammatory and genotoxicity potentials of the fractions of *Archidium ohioense* (Schimp.Ex Mull) extract. Ife J. Sci. 2018;20(3):487-496
14. Azeez SO, Ayo-Lawal RA, Olawuni IJ, Abraham OG. Evaluation of bio-toxicity and cytogenetic effect of methanolic leaf extract of *Heliotropium indicum* L. Int. J. Biol. Chem. Sci.2020;14(1):1-10. Available:https://dx.doi.org/10.4314/ijbcs.v14i1.1
15. Wu, C. An important player in brime shrimp lethality bioassay: The solvent. J. adv. Pharm. Technol. Res. 2014;5: 57-58.
16. Umaru, IJ, Badruddin, FA, Assim, ZB, Umaru, HA. Cytotoxicity (Brime shrimp lethality bioassay) of *Barringtonia rocemosa* leaves, stem-bark and root extracts. *J. Biotechnol. Bioeng.*2018; 2(2):45-50
17. Waghulde, S, Kale, MK, Patil, VR. Brine shrimp lethality assay of the aqueous and

ethanolic extracts of the selected species of medicinal plants. Proceeding. 2019; 41(1):47.
Available:https://doi,org/10.3390/ecsoc-23-06703

18. Trease G, Evans SM. *Pharmacognosy*. 5th Edition, Bailer Tindal London. 2002;23- 67.
19. Sofowora, A. Screening plants for bioactive agents. In: Medicinal Plants and Traditional Medicine in Africa. Third Edition, Spectrum Books Limited, Ibadan, Nigeria.2006;150-153.
20. Namita K, Somia S. *Allium cepa* root chromosomal aberration assay: A review. Indian J. Pharm. Biol. Res. 2013; 1(3):105-119.
21. Fiskesjo G. The *Allium* test as a standard in environmental monitoring. Hereditas. 1985;102: 99–112.
22. Grant WF. Chromosome aberration assays in *Allium.* A report of U.S. Environmental Protection Agency Gene- Tox Program. Mutation Research. 1982;99:273-291.
23. El-Ghamery AA, El-Nahas AI, Mansour MM. The action of atrazine herbicide as an indicator of cell division on chromosomes and nucleic acids content in root meristems of *Allium cepa* and *Vicia faba*. Cytologia. 2000;65:277-287.
24. Sadia KB, Vahidy AA. Cytotoxic effects of herbicide ronstar on meristematic cells of *Allium cepa* L. Pak. J. Bot.1994;26: 69-74.
25. Zabka, A, Winnick, K, Polit, JT, Bernasinska-Stomezewska, J, Maszecoski, J. 5-Aminouracil and other inhibitors of DNA replication induced bophasic interphase-mitotic cells in apical meristem of *Allium cepa.* Plant Cell Reports 2020;39:1013-1028.
Available: https://doi.org/10.1007/s00299-020-02545-9
26. Inoue N, Terabayashi T, Kawashima YT, Fujinami D, Matsuoka S, Kawano M, Tanaka K, Tsumura H, Ishizaki T, Narahara H, Kohda D, Nishida KY, Hanada K. The benzylisoquinolinealkaloids, berberine and coptisine, act against camptothecin-resistanttopoisomeraseImutants. Scientific Report. 2021;(2021)11.7718.
Available:https.//doi.org/10.10381/s4198-021-87344-2
27. Jain AK, Andsorbhoy, RK. Cytogenetical studies on the effects of some chlorinated pesticides III. Cytologia. 1988;53:427-436.
28. Hidalgo A, Gonzalez-Reyes, JA, Navas, P, Garcia-Herdugo G. Abnormal mitosis and growth inhibition in *Allium cepa* roots induced by propham and chlorpropham. Cytobios. 1989;57:7-14.
29. Celik, TA. Potential genotoxic and cytotoxic effects of plant extracts. *In: A compendium of essays on alternative therapy.* Bhattacharya A. (ed). Intechopen Bool Series. ISBN: 978-953-51-6731-0. 2012. Pp 233-250
30. Al-Faifi, ZIA, Masrahi, YS, Aly, MS, Al-Turki, TA, Dardeer,T. Evaluation of cytotoxic and genotoxic effects of *Euphorbia triaculeata* Forssk. Extract. Asian Pac. J. Cancer Prev. 2017; 18(3): 771-777.
DOI: 10.22034/APJCP.2017.18.3.771
31. Moshi MJ, Mbwambo ZH, Nondo RSO, Masimba PJ, Kamuhabwa, A, Kapingu, MC, Thomas, P, Richard, M. Evaluation of ethnomedical claims and brine shrimp toxicity of some plants used in Tanzania as traditional medicines. Afr. J. Tradit. Complement. Altern. Med. 2006;3:48 - 58.
32. McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. *Drug Inf. J.* 1998;32:513-524
33. Ratnayake, R, Covell, D, Ransom, TT, Gustafson, KR, Beutler, JA. Englerin A - a selective inhibitor of renal cancer cell growth, from *Phyllanthus engleri*. *Organic Letters.* 2009;11: 57-60.
34. Goodman LS, Gilman AG, Gilman A. Antiproliferative agents and immunosuppressive drugs. *In: The Pharmacological basis of therapeutics*. Macmillan Publishing Co., Inc., USA, 6th edition.1980;1299-1313.
35. Nyamai, DW, Arika, W, Ogola, PE, Njagi, ENM, Ngugi, MP. Medicinally important phytochemicals: An untapped research avenue. *Pharmacogn. Phytochem.* 2016;4(1): 35-49
36. Bose, J, Malik, J, Manda, SC. Application of phytochemicals in pharmaceuticals. *In: Advances in Pharmaceutical Biotechnology*. Patra J., Shukla, A. and Das, G. (eds). Springer, Singapore. 2020
Available:https://doi.org/10.1007/978-981-15-2195-9_5
37. Abraham J. Phytochemicals and their roles in Pharmaceuticals. *In Advances in Pharmaceutical Biotechnology*. Patra J, Shukla A, Das G. (eds). Springer, Singapore;2020.
Available:https://doi.org/10.1007/978-981-15-2195-9_16

38. Selvakumar, P, Kaniakumari, D, Loganathan, V. Phytochemical screening and antioxidant activity of red flowered *Mirablis jalapa* leaf in different solvent. Int. J. Pharma Bio Sci. 2012;3(4): 440-446.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/75867