Toxicology Reports 7 (2020) 671–679

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Evaluation of the biosafety potentials of methanol extracts/fractions of *Tapinanthus bangwensis* and *Moringa oleifera* leaves using *Allium cepa* model

Godwin O. Ihegboro[a,*], Adamu J. Alhassan[b], Chimaobi J. Ononamadu[a], Tajudeen A. Owolarafe[a], Mohammed S. Sule[b]

[a] Department of Biochemistry and Forensic Science, Faculty of Sciences, Nigeria Police Academy, Wudil, P.M.B 3474 Kano-Maiduguri Road, Kano, Nigeria
[b] Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University, Kano, Nigeria

ARTICLE INFO

Keywords:
Cytotoxicity
Allium cepa
Tapinanthus bangwensis
Moringa oleifera
Mitotic Index
Chromosomal aberration

ABSTRACT

Medicinal plants are considered two-edged swords. They are pharmacologically beneficial and sometimes toxic when abused. The health benefit of medicinal plant is due to their abundant phytochemical endowment. The present study is aimed at evaluating the biosafety potentials of methanol extracts/fractions of *Tapinanthus bangwensis* (*T. bangwensis*) and *Moringa oleifera* (*M. oleifera*) leaves using *Allium cepa* (*A. cepa*) model. Cytotoxicity assay was carried out by *A. epa* model. The cytotoxicity parameters studied were number of dividing cells, percentage mitotic index (% MI), root tip growth length and chromosomal aberrations. The phytochemistry was determined by UV-spectrophotometry while 2,2-diphenyl-1-picrylhydrazine (DPPH) and nitric oxide (NO) were used to assay antioxidant activity. The *A. cepa* assay result showed that the inhibitory effect of *M. oleifera* on root tip growth length was higher compared to *T. bangwensis* at 60-100mg/100ml concentrations. Decreased number of dividing cells and percentage mitotic index as concentrations increased observed indicate cytotoxicity however the acetone fractions were most cytotoxic. The frequently occurring chromosomal aberrations observed were vagrant, bridged, attached and sticky chromosomes while C-mitosis and binuclear chromosomes were not observed. Therefore cytotoxic effect was significant at 100mg/100ml. The phytochemical screening of methanol extracts/fractions of *T. bangwensis* and *M. oleifera* leaves revealed the presence of alkaloids, flavonoids, phenolics, saponins and tannins however saponins were significantly abundant in both plants compared to others. Alkaloid content was found to be low but was not detected in the acetone fractions of the plants. Methanol extracts/fractions of the plants' leaves exhibited antioxidant activities but was more prominent in the ethylacetate fraction of *T. bangwensis* and acetone fraction of *M. oleifera*. In conclusion, administration at 100mg/100ml could be unsafe for the biological system.

1. Introduction

In time immemorial, man has depended on herbs as supplements for body preservation as well as therapy for several diseases [1,2] with little or no knowledge of its toxic effect especially in developing countries like Nigeria [3]. Medicinal plants play a vital role in novel drug discovery used in modern medicines for the treatment and management of various diseases [4]. According to World Health Organisation (WHO) about 80% of the world's population rely on phytomedicine for the source of their healthcare [5,6]. The interest in herbal preparation/products worldwide is fast gaining acceptance and is fuelled by the increasing cost implication of synthetic drugs and their potential adverse side effects [7]. Cytotoxicity study is a useful tool used in drug design and development for the evaluation of the biosafety characteristic of substances including plant extracts or bioactive compounds isolated from plants. Despite plants being a rich source of useful chemical compounds with different pharmacological benefits on biological systems [8,9] some may be toxic in nature. There are some medicinal plants toxicity-associated problems such as allergic reactions, gastrointestinal irritation, haemolysis, organ damage and carcinogenicity have been reported [10–12], therefore, scientific evidence regarding the efficacy and safety of herbal products should be established before they are developed into drugs. *T.bangwensis* (African mistletoe) is a host-dependent plant belonging to the Loranthaceae family. It is also called “all heal tree, bird lime or tree of life”. Traditionally, in Nigeria, the Hausas call it *Kauci (Kanchi)*, Yoruba-*Afomo onisana* and Igbo –*Apari*

* Corresponding author.
E-mail addresses: goihegboro@polac.edu.ng (G.O. Ihegboro), ajalhassan.bch@buk.edu.ng (A.J. Alhassan), ononamaducj@polac.edu.ng (C.J. Ononamadu), taowolarafe@polac.edu.ng (T.A. Owolarafe), mssule.bch@buk.edu.ng (M.S. Sule).

https://doi.org/10.1016/j.toxrep.2020.05.001
Received 3 September 2019; Received in revised form 2 May 2020; Accepted 6 May 2020

or Awurusie. It obtains its water and mineral nutrients from the host plant and is photosynthetically active [13]. In Nigeria and other parts of the world extracts from the leaves of *T. bangwensis* are used in the treatment and management of various ailments such as anticancer agents [14], anti-inflammatory agent and diabetes mellitus but to mention a few [15,16]. *M.oleifera* is also called "Drumstick tree, Horseradish tree, benzoil tree, mother's best friend or miracle tree" [17]. In Nigeria, the Hausas call it *Bagaruar maka (masar), Rimin nacara (turawa), Koraukin, Zaila, Dango, Barambo* or popularly known as *Zogale*; Yoruba *-Adagba malero, Ewe igable Ewe ile, Ewe igbale, Idagbo monoye* (tree that grows crazily), Igbo - *Odudu oyibo, Okwe oyibo, Okwe olu, Okochi egbu* (cannot be killed by drought). *M.oleifera* is a fast-growing drought-resistant and deciduous tree belonging to the Moringaceae family and possesses impressive medicinal uses with higher nutritional values [18]. *M.oleifera* has been found to be anti-hypertensive [19], anti-inflammatory, antimicrobial anticancer, anti-diabetic and antioxidant agents [20]. Despite the natural state of these medicinal plants, previous studies provide information on the pharmacological effect of these plants, however scanty information on the safety effect of *T.bangwensis* and *M.oleifera* is known. Therefore the present study is aimed at evaluating the biosafety potentials of *T.bangwensis* and *M.oleifera* as medicinal plants for human use using *A.cepa* model.

2. Materials and methods

2.1. Preparation of plant materials

Fresh leaves of *T.bangwensis* (Engl & K.Krause) Danser were obtained in Lagos and were botanically identified in the Department of Pharmagnosy, University of Lagos. The plant voucher number (LUH 3856) was deposited. *M.oleifera.* Lam leaves were obtained from Nigeria Police Academy, Wudil, Kano and botanically identified in the Department of Botany, Bayero University Kano and voucher number (BUH 4123) was deposited in the herbarium. The leaves of the plants were washed and air-dried for 5 days under dry climatic condition.

2.2. Extraction of plant materials

About 2.15 kg powdered leaves each of *T.bangwensis* and *M.oleifera* was extracted with 10 l of 100% methanol solvent using maceration method. The filtrates were then concentrated by evaporation at room temperature until a constant weight was obtained. The mass yield of methanol extract of *T.bangwensis* (MeCE 1) and *M.oleifera* (MeCE 2) were 187.5 g (8.71%) and 209.0 g (9.73%), respectively.

2.3. Solvent-partition extraction of plant extracts

The method of Farahziela et al. [21] was adopted. The principle of operation is based on difference in density gradient. The solvent partition was carried out using three different solvent polarities such as ethylacetate, acetone and water. The solution mixture for the extraction was ethylacetate-water and acetone-water in 3:2, respectively. About 60 g of the methanol extract of *T.bangwensis* was dissolved in methanol to obtain a solution. The resulting solution was poured into the separatory funnel, shaken and allowed to stand for 2 h. The ethylacetate layer was collected, dried and labelled as ETF 1 (14.45 g). About 60 g of methanol extract of *T.bangwensis* was dissolved in methanol and was poured into the separatory funnel, shaken and allowed to stand. The acetone portion was collected, dried and labelled as ACF 1 (9.37 g). The same procedure as above was followed for *M.oleifera* and the mass yield of ethylacetate fraction (ETF 2) and acetone fraction (ACF 2) were 8.02 g and 7.45 g, respectively.

2.4. Phytochemical compound assays

The alkaloid content was estimated gravimetrically using the method of Harbourne [22]. Flavonoid content was estimated spectrophotometrically by the method described by Chang, et al., [23], Phenolic content was estimated spectroscopically by Folin-Ciocalteu reagent method described by Slinkard and Singleton [24]. Spectrophotoscopic estimation of tannin was based on the method of Van-Burden and Robinson [25] while Saponin content was estimated gravimetrically by the method of Obadoni and Ochuko [26].

2.5. In vitro antioxidant activity

The free radical scavenging activity of the methanol extracts/fractions of *T.bangwensis* and *M.oleifera* based on the scavenging of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was estimated according to the method described by Burtis and Bucar [27] while the nitric oxide assay was determined by the method described by Alisi and Onyeze [28].

2.6. Cytotoxicity study using Allium cepa

The method of Fiskesjo [29] was followed. Onion bulbs (*Allium cepa*) were placed on a container filled with distilled water for 24 h and subsequently series of five best growing bulbs were exposed to the test solutions (10, 40, 60, 80 and 100 mg/100 mL) for 4 days in the dark and at room temperature. Distilled water was used as the control. The root tip growth lengths were then measured 24 hourly using a ruler. For chromosomal aberration evaluation, the root tips (5 − 7 mm) from the bulb were excised and fixed in ethanol-glacial acetic acid solution (3:1 v/v). The collected root tips were then hydrolysed in 1 N hydrochloric acid at 60 °C for 6 min and then rinsed with distilled water. The root tip was squashed in a glass slide and then stained with 2% aceto-orcein for 10 min. and covered carefully with a slide cover to avoid air bubbles. The slides were then sealed with fingernail polish. Ten slides were prepared for each concentration and the control and then viewed under a microscope with high resolution mounted on a camera. The mitotic index was then calculated.

3. Results

Methanol extracts/fractions of *T.bangwensis* and *M.oleifera* were extracted and phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, saponins and tannins (Table 1).The methanol extract of *T.bangwensis* (MeCE 1) showed the most abundant alkaloid content while ethylacetate fraction of *M.oleifera* (ETF 2) showed the lowest alkaloid content. Alkaloid was not detected in the acetone fractions of *T.bangwensis* and *M.oleifera* (ACF 1 and ACF 2).The flavonoid content was high in MeCE 1, ETF 1 and MeCE 2 but low in ACF 1, ETF 2 and ACF 2.The result showed that MeCE 2 and ETF 2 possessed significant levels of phenolics compared to other samples The result also revealed that the methanol extracts/fractions of the plant species contained significant levels of saponins compared to other phytochemical compounds. From the result obtained *T.bangwensis* possessed more tannins compared to *M.oleifera*. (Table 1)

The inhibitory activity of the methanol extracts/ fractions (IC_{50}) of the plants were calculated. The DPPH result showed that ETF 1 exhibited the strongest antioxidant activity (IC_{50} = 3.49 ± 0.04 μg/mL) compared to ascorbic acid (control) (IC_{50} = 3.75 ± 0.37 μg/mL) while the lowest antioxidant activity was observed in ACF 1 (IC_{50} = 5.84 ± 0.22 μg/mL) (Table 2). For Nitric oxide assay, ACF 2 exhibited the highest antioxidant activity (IC_{50} = 3.28 ± 0.39 μg/mL) while the lowest antioxidant activity was observed in ACF 1 (IC_{50} = 4.58 ± 0.87 μg/mL) (Table 3).

The cytotoxic effects of the methanol extracts/fractions of *T.bangwensis* and *M.oleifera* were studied using *A.cepa* model [23]. The

Table 1
Levels of phytochemical compounds in methanol extracts/fractions of *T.bangwensis* and *M. oleifera*.

Extracts/Fractions	Alkaloids (mg/100 g)	flavonoids (mg/100 g)	phenolics (mg/100 g)	saponins (mg/100 g)	tannins (mg/100 g)
MeCE 1	13.08 ± 0.05	27.26 ± 0.40	27.98 ± 0.14	51.82 ± 0.90	21.93 ± 0.20
ETF 1	12.01 ± 0.13	21.41 ± 0.30	29.03 ± 0.15	49.45 ± 0.46	19.74 ± 0.23
ACF 1	ND	04.12 ± 0.08	25.47 ± 0.15	48.23 ± 0.60	18.57 ± 0.20
MeCE 2	14.01 ± 0.15	25.92 ± 0.28	39.93 ± 0.26	54.57 ± 0.53	10.43 ± 0.64
ETF 2	10.71 ± 0.18	10.23 ± 0.28	36.45 ± 0.24	51.51 ± 0.57	06.87 ± 0.21
ACF 2	ND	06.35 ± 0.01	24.76 ± 0.15	40.12 ± 0.68	04.96 ± 0.16

Values were in triplicates and were expressed statistically as Mean ± SD. **Keys:** MeCE 1 = Methanol extract of *T.bangwensis*, ETF 1 = Ethylacetate fraction of *T.bangwensis*, ACF 1 = Acetone fraction of *T.bangwensis*, MeCE 2 = Methanol extract of *M.oleifera*, ETF 2 = Ethylacetate fraction of *M.oleifera* ACF 2 = Acetone fraction of *M.oleifera* ND = Not detected.

Table 2
DPPH Antioxidant Activity of Methanol Extracts/Fractions of *T.bangwensis* and *M.oleifera*.

Conc/samples	25 (µg/mL)	50 (µg/mL)	100 (µg/mL)	150 (µg/mL)	200 (µg/mL)	250 (µg/mL)	IC_{50} (µg/mL)
Ascorbic acid	42.29 ± 0.40^a	51.33 ± 0.41^a	57.36 ± 0.76^a	63.74 ± 0.30^a	71.32 ± 0.50^a	83.78 ± 0.72^a	3.75 ± 0.37^a
MeCE 1	20.50 ± 0.16^b	39.73 ± 0.43^b	50.93 ± 0.28^a	58.61 ± 0.24^a	63.33 ± 0.29^a	67.56 ± 0.23^b	4.47 ± 0.16^b
ETF 1	18.86 ± 0.45^b	41.83 ± 0.43^b	68.52 ± 0.32^a	70.20 ± 0.75^a	73.06 ± 0.60^a	79.36 ± 0.26^a	3.49 ± 0.04^a
ACF 1	19.96 ± 0.21^b	36.70 ± 0.17^b	39.78 ± 0.35^b	45.52 ± 0.29^b	49.07 ± 0.21^b	53.88 ± 0.19^b	5.84 ± 0.22^b
MeCE 2	21.17 ± 0.20^b	27.64 ± 0.40^b	50.11 ± 0.28^a	56.71 ± 1.85^a	53.31 ± 0.42b	66.81 ± 0.46^b	4.52 ± 0.04^b
ETF 2	17.57 ± 0.31^b	40.87 ± 0.37^b	50.27 ± 0.41^a	55.15 ± 0.41^a	57.30 ± 0.75^b	58.10 ± 0.43^b	5.06 ± 0.63^b
ACF 2	24.60 ± 0.28^b	39.18 ± 0.37^b	59.11 ± 0.30^a	66.07 ± 0.13^a	66.87 ± 0.29^a	76.56 ± 0.21^a	4.00 ± 0.18^a

Values were in triplicates and were expressed statistically as Mean ± SD.. Values bearing the same superscript down the column are not statistically significant difference at $p < 0.05$. **Keys**: MeCE 1 = Methanol extract of *T.bangwensis,* ETF 1 = Ethylacetate fraction of *T.bangwensis,* ACF 1 = Acetone fraction of *T.bangwensis,* MeCE 2 = Methanol extract of *M.oleifera,* ETF 2 = Ethylacetate fraction of *M.oleifera* and ACF 2 = Acetone fraction of *M..oleifera.*

Table 3
Nitric oxide Antioxidant Activity of Methanol Extracts/Fractions of *T.bangwensis* and *M. oleifera*.

Conc/Samples	25 (µg/mL)	50 (µg/mL)	100 (µg/mL)	150 (µg/mL)	200 (µg/mL)	250 (µg/mL)	IC_{50}(µg/mL)
Ascorbic acid	41.60 ± 0.65^a	51.27 ± 0.42^a	56.78 ± 0.50^a	63.78 ± 0.29^a	72.33 ± 0.55^a	78.27 ± 0.78^a	3.81 ± 0.83^a
MeCE 1	22.72 ± 0.48^b	52.55 ± 0.78^a	55.42 ± 0.21^a	72.27 ± 0.55^a	73.27 ± 0.42^a	79.33 ± 0.55^a	3.87 ± 0.54^a
ETF 1	29.19 ± 0.34^b	55.05 ± 0.41^a	59.70 ± 0.36^a	75.09 ± 0.21^a	81.51 ± 0.29^b	83.42 ± 0.42^a	3.56 ± 0.31^a
ACF 1	22.13 ± 0.27^b	39.66 ± 0.78^a	52.96 ± 0.48^a	60.35 ± 0.59^a	63.57 ± 0.49^a	67.17 ± 0.42^b	4.58 ± 0.87^b
MeCE 2	32.06 ± 0.69^b	48.86 ± 0.48^a	64.43 ± 0.28^b	80.33 ± 036^b	81.97 ± 0.28^b	84.34 ± 0.28^a	3.64 ± 0.67^a
ETF 2	32.24 ± 0.36^b	47.36 ± 0.89^a	61.93 ± 0.42^b	77.10 ± 0.48^b	79.10 ± 0.21a	83.01 ± 0.42^a	3.67 ± 0.95^a
ACF 2	21.67 ± 0.34^b	48.86 ± 0.93^a	52.41 ± 0.42^a	69.85 ± 0.62^a	81.37 ± 0.34^b	82.69 ± 0.21^a	3.28 ± 0.39^a

Values were in triplicates and were expressed statistically as Mean ± SD. Values bearing the same superscript down the column are not statistically significant at $p < 0.05$. **Keys**: MeCE 1 = Methanol extract of *T.bangwensis,* ETF 1 = Ethylacetate fraction of *T.bangwensis,* ACF 1 = Acetone fraction of *T. bangwensis,* MeCE 2 = Methanol extract of *M. oleifera,* ETF 2 = E thylacetate fraction of *M. oleifera* and ACF 2 = Acetone fraction of *M. oleifera.*

Table 4
Effects of Methanol Extracts/Fractions of *T.bangwensis* and *M.oleifera* on root tip growth length on *Allium cepa.*

Conc/Samples	20 mg/100 mL	40 mg/100 mL	60 mg/100 mL	80 mg/100 mL	100 mg/100 mL
MeCE 1	4.80 ± 1.15	3.58 ± 0.55	3.43 ± 0.45	3.37 ± 0.29	3.07 ± 0.28
ETF 1	3.98 ± 0.82	3.75 ± 0.90	3.48 ± 0.30	3.41 ± 0.37	3.30 ± 0.50
ACF 1	3.41± 0.28	3.12 ± 0.45	2.35 ± 0.33	2.17 ± 0.16	1.07 ± 0.30
MeCE 2	3.42 ± 0.24	2.52 ± 0.23	1.10 ± 0.11	0.75 ±0.05	0.58 ± 0.11
ETF 2	3.03 ± 0.34	2.51 ± 0.35	2.43 ± 0.44	1.65 ± 0.44	0.93 ± 0.25
ACF 2	2.48±0.28	2.17±0.38	1.98 ± 0.12	0.83±0.25	0.78±0.16

Values were in triplicates and were expressed statistically as Mean ± SD. **Keys:** MeCE 1 = Methanol extract of *T. bangwensis*.
ETF 1 = Ethylacetate fraction of *T. bangwensis*, ACF 1 = Acetone fraction of *T. bangwensis*, MeCE 2 = Methanol extract of *M. oleifera,* ETF 2 = Ethylacetate fraction of *M. oleifera*, ACF 2 = Acetone fraction of *M. oleifera.*

result showed decrease in root tip growth length as concentration increased. At the highest concentration (100 mg/100 mL), MeCE 2 exhibited the highest root growth inhibition while at the lowest concentration (20 mg/100 mL) acetone fraction of *M.oleifera* (ACF 2) exhibited the highest inhibitory effect (Table 4). Tables 5 and 6, showed decreased percentage mitotic index (% MI) as concentration increased. ACF 1 and ACF 2 were the lowest 5.17% and 4.95%, respectively. This indicates that acetone fractions of the plants are most cytotoxic, followed by methanol extracts MeCE 1(6.78%) and MeCE 2 (6.84%) and the lowest is ethylacetate fractions ETF 1 (7.03%) and ETF 2 (7.06%), respectively. Tables 5 and 6, showed significant increase in sticky chromosomes however, acetone fraction of *M.oleifera,* was significantly higher *(*ACF 2), followed by bridged and vagrant chromosomes while attached and multipolar chromosomes occurred the least. C-mitosis and Binuclear chromosomes were not observed. The percentage chromosomal aberrations for all the samples were found to be less than 4.19% at the highest concentration. The various chromosomal aberrations observed are shown in (Figs. 2–7) while Fig. 1 showed normal mitotic division.

Table 5
Cytological effect of Methanol extracts/fractions of *T. bangwensis* at different concentrations using *Allium cepa*.

	Parameters/ concentrations	No of cells	P	M	A	T	No of dividing cells	% MI	Sticky	C-mitosis	Bridged fragment	Vagrant	Binuclei	Multipolar anaphase	Attached	Total CA	% CA
Methanol	Control	500	3	16	11	17	47	9.40	0	0	0	0	0	0	0	0	0.00
	20 mg/100 mL	481	5	18	13	15	51	10.60	10	0	8	8	0	0	5	31	6.44
	40 mg/100 mL	475	7	15	12	14	48	10.11	9	0	7	5	0	0	5	26	5.47
	60 mg/100 mL	469	3	14	12	14	43	9.17	8	0	7	5	0	0	3	23	4.90
	80 mg/ 100 mL	463	3	16	8	10	37	7.99	6	0	5	6	0	0	4	21	4.54
	100 mg/100 mL	457	0	10	9	12	31	6.78	6	0	5	4	0	1	2	18	3.94
Ethylacetate	Control	500	3	16	11	17	47	9.40	0	0	0	0	0	0	0	0	0.00
	20 mg/100 mL	465	6	15	11	14	46	9.89	9	0	6	5	0	0	5	25	5.38
	40 mg/100 mL	459	3	14	12	13	42	9.15	8	0	6	5	0	0	4	23	5.23
	60 mg/100 mL	453	4	12	10	12	38	8.38	7	0	5	6	0	0	2	20	4.42
	80 mg/100 mL	447	1	10	12	11	34	7.61	6	0	7	5	0	0	0	18	4.03
	100 mg/100 mL	441	0	13	10	8	31	7.03	4	0	5	4	0	0	3	16	3.63
Acetone	Control	500	3	16	11	17	47	9.40	0	0	0	0	0	0	0	0	0.00
	20 mg/100 mL	469	2	16	11	15	44	9.38	10	0	6	6	0	0	5	27	5.76
	40 mg/100 mL	463	3	14	10	14	41	8.86	8	0	6	7	0	0	4	25	5.40
	60 mg/100 mL	459	0	13	10	12	35	7.63	7	0	5	5	0	0	3	20	4.36
	80 mg/100 mL	451	0	11	8	10	29	6.43	6	0	5	7	0	0	0	18	3.99
	100 mg/100 mL	445	0	9	5	9	23	5.17	6	6	0	3	5	0	0	17	3.82

Keys: P = Prophase, M = Metaphase, A = Anaphase, T = Telophase, MI = Mitotic index, CA = Chromosomal aberrations.

Table 6
Cytological effect of Methanol extracst/fractions of *M. oleifera* at different concentrations using *Allium cepa*.

	Parameters/ concentrations	No of cells	P	M	A	T	No of dividing cells	% MI	sticky	C-mitosis	Bridged fragment	Vagrant	Binuclei	Multipolar anaphase	Attached	Total CA	% CA
Methanol	Control	500	3	16	11	17	47	9.40	0	0	0	0	0	0	0	0	0.00
	20 mg/100 mL	472	2	21	12	14	49	10.38	8	0	7	9	0	0	6	30	6.36
	40 mg/100 mL	467	5	17	10	14	46	9.85	9	0	6	8	0	0	4	27	5.78
	60 mg/100 mL	462	3	15	10	13	41	8.87	8	0	4	6	0	0	4	22	4.76
	80 mg/100 mL	459	0	14	10	13	37	8.06	8	0	6	5	0	0	4	23	5.01
Ethylacetate	100 mg/100 mL	453	1	12	8	10	31	6.84	7	0	4	5	0	0	3	19	4.19
	Control	500	3	16	11	17	47	9.40	0	0	0	0	0	0	0	0	0.00
	20 mg/100 mL	457	0	16	11	16	43	9.41	10	0	7	7	0	0	4	28	6.13
	40 mg/100 mL	453	2	14	10	13	39	8.61	8	0	7	6	0	0	3	24	5.30
	60 mg/100 mL	447	1	13	10	11	35	7.83	7	0	6	4	0	1	5	23	5.15
	80 mg/100 mL	441	0	12	8	11	31	7.03	7	0	6	5	0	0	3	21	4.76
	100 mg/100 mL	439	2	10	9	10	31	7.06	6	0	5	4	0	0	2	17	3.81
Acetone	Control	500	3	16	11	17	47	9.40	0	0	0	0	0	0	0	0	0.00
	20 mg/100 mL	465	0	21	13	16	50	10.75	11	0	8	12	0	0	4	35	7.53
	40 mg/100 mL	463	3	18	12	14	47	10.15	10	0	8	10	0	0	4	32	6.91
	60 mg/100 mL	459	2	14	10	15	41	8.93	10	0	4	5	0	1	3	23	5.01
	80 mg/100 mL	443	1	13	4	9	27	6.09	9	0	3	6	0	1	2	21	4.70
	100 mg/100 mL	424	0	7	4	10	21	4.95	7	0	3	3	0	0	3	16	3.77

Keys: P = Prophase, M = Metaphase, A = Anaphase, T = Telophase, MI = Mitotic index, CA = Chromosomal aberrations.

4. Discussion

The present study evaluated the amount of phytochemical compounds, antioxidant activity and biosafety potentials of methanol extracts/fractions of *T.bangwensis* and *M.oleifera* using *Allium cepa* model. Phytochemical substances which are key components of medicinal (or herbal) plants elucidate pharmacological and physiological effects on biological systems [30]. The antioxidant activity exhibited by the plants may be due to the significant levels of saponins, phenolics, alkaloids and flavonoids content [31–34]. During drug design and development, biosafety is of great importance. Toxicological studies provide an insight into the safety or tolerability of the drug/extracts in the biological system. Several toxicological models are employed for evaluating drug/extract biosafety such as brine shrimp, *A.cepa*, cell lines and animals. However, *A cepa* model provides information on the effect of toxic substance at molecular level (or chromosome architecture) called chromosomal aberration. Chromosomal aberrations are deformations observed on chromosomes due to mutational conformation caused by the influence of mutagens such as ultraviolet rays, toxic chemicals, drugs/herbal products, phytochemical compounds [35]. From the cytotoxicity result obtained the extracts/fractions of *T.bangwensis* and *M.oleifera* showed cytotoxicity characterized by decrease in percentage mitotic index, number of dividing cells and root tip growth length as concentration increased [36–40]. The mitotic index is an acceptable tool used to measure the proportionality of cells in the metaphase (M-phase) of the cell cycle and its inhibition could be interpreted as cellular death, delay in cell proliferation kinetics or cell damage [41,35,42]. A decrease in mitotic index compared to the control indicates inhibitory and mitodepressive effects [43] while an increase in mitotic index indicate increase in cell division/cell proliferation [44].The inhibitory effect observed in this study could be due to either chromatin dysfunction, disturbance in the cell cycle induced by the interaction of the phytochemicals with the DNA, inhibition of DNA synthesis or complete arrest of mitotic cycle at the G2 phase preventing the cell from entering mitosis [45–48]. Mitodepression on the other hand occur in the interphase nucleus and indicate the ability of toxic substances to block DNA

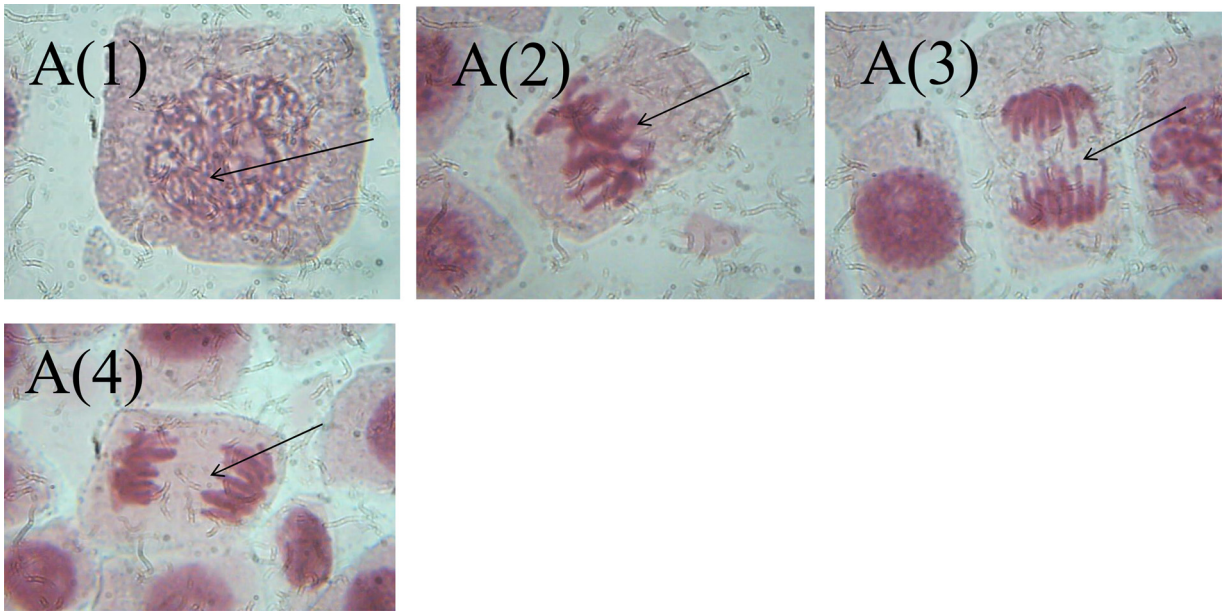

Fig. 1. Photomicrographs showing normal mitotic division A(1) Prophase A(2) Metaphase A(3) Anaphase A(4) Telophase.

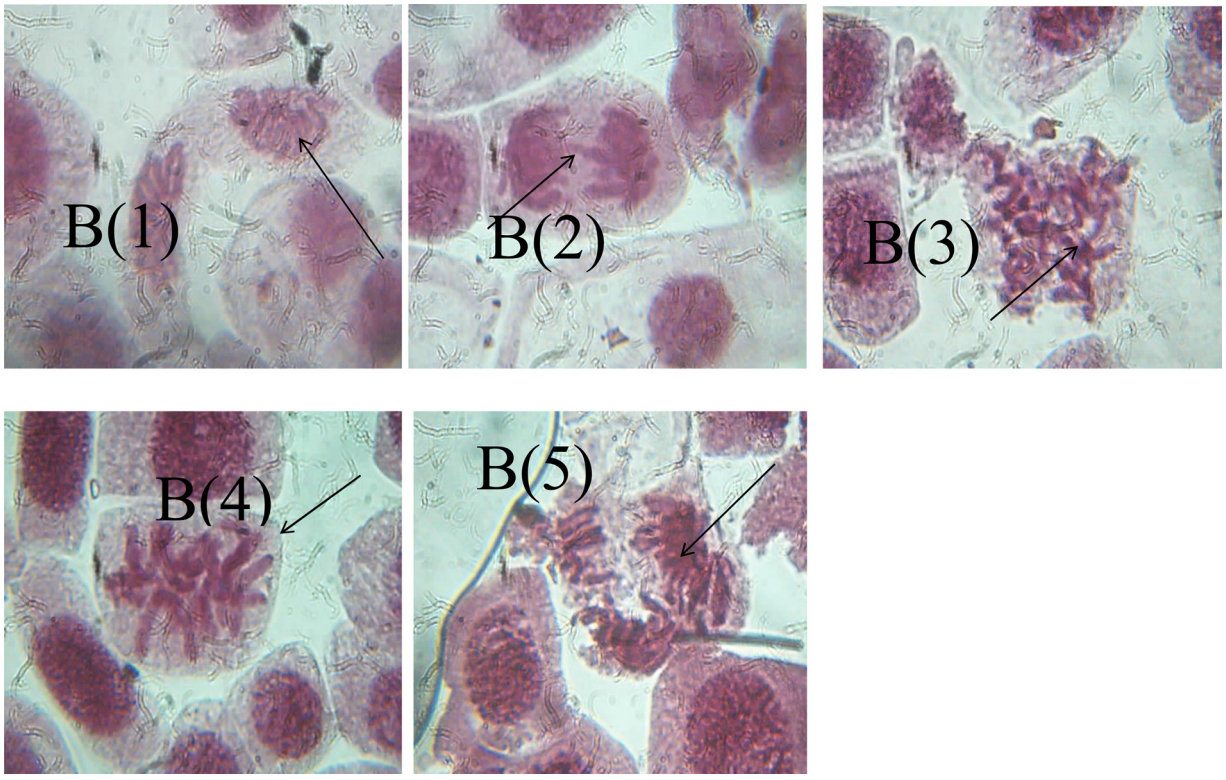

Fig. 2. Photomicrographs showing the effect of methanol extract of *T.bangwensis* B(1) Vagrant B(2) Bridged telophase B(3) Bridged and fragmentation B(4) Attached B(5) Multipolar.

and nucleus-protein biosynthesis [36,49]. The inhibitory action of plant extracts is the mechanism (or mode of action) utilized in the management of cancer traditionally and evidence supporting this phenomenon has been documented on some plant extracts with anticancer therapy [50,51]. Chromosomal aberrations (CA) which are changes in the chromosome architecture (or structure) result from either a break or exchange of chromosomal materials. It could also occur due to spindle failure orchestrated by the interaction of phytochemical constituents with the spindle apparatus, for instance, alkaloids have been reported to inhibit mitosis and also bind to tubulin preventing the formation of the mitotic spindle [52]. This suggests that the alkaloids present in the plants could be responsible for the aberrations observed. Sticky, bridged, vagrant and attached chromosomes were predominant while there was no C-mitosis and binuclear chromosomes observed. However, sticky chromosome occurred significantly compared to other chromosomal aberrations and this indicates cytotoxic potential of the plant extracts/fractions [42]. Stephen mentioned that stickiness is a physical adhesion that involves mainly the proteinaceous matrix of the chromatin material and occur as a result of depolymerisation of DNA, partial dissolution of nucleoproteins, breakage and exchanges of the basic

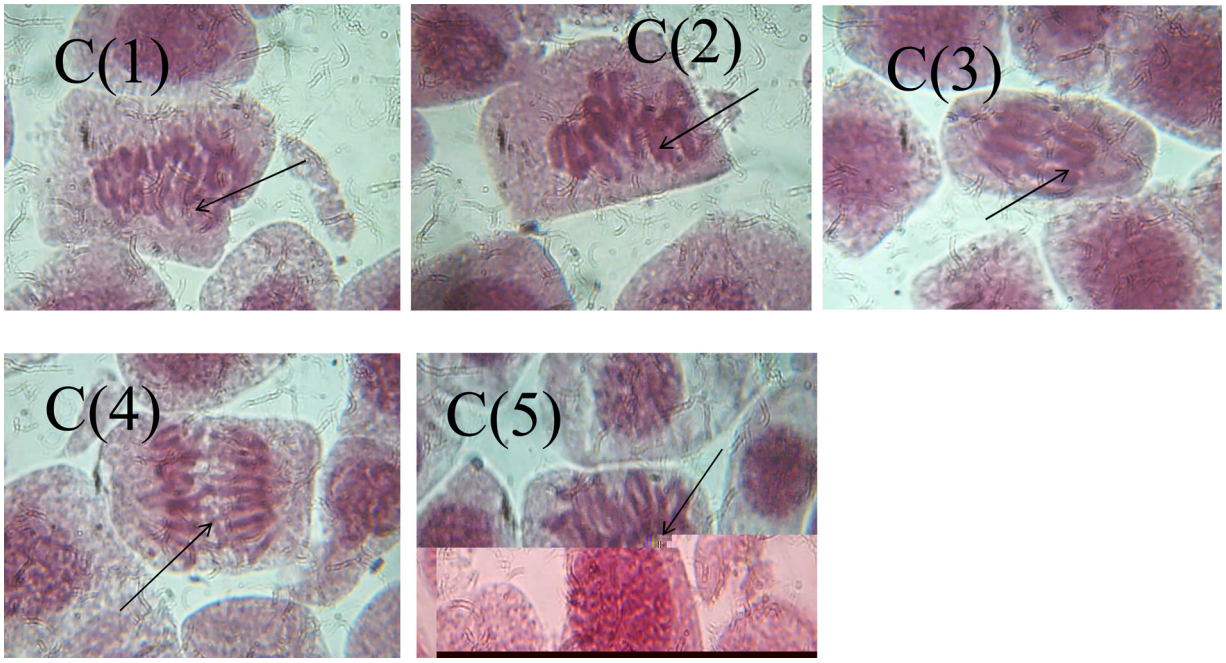

Fig. 3. Photomicrographs showing the effect of ethylacetate fraction of *T.bangwensis* C(1) Bridged C(2(Vagrant C(3) Bridged telophase. C(4) Attached C(5) Sticky.

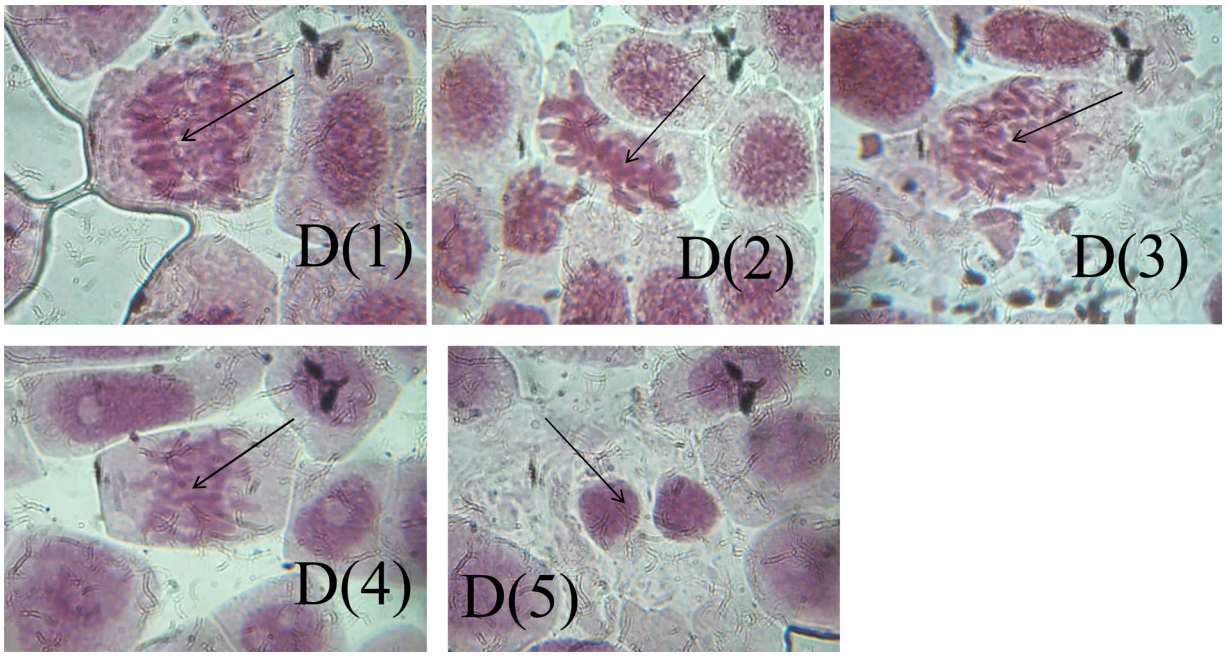

Fig. 4. Photomicrographs showing the effect of acetone fraction of *T.bangwensis* D(1) Bridged anaphase D(2) Vagrant D(3) Attached D(4) Bridged and fragmentation D(5) Sticky.

folded fibre units of chromatids and stripping of the protein covering DNA in chromosomes [53,54]. This could be attributed to the effect of chemicals/extracts on the physicochemical properties of DNA, protein or both, on the formation of complexes with phosphate groups in DNA, on DNA condensation or on formation of inter and intra chromatid cross links [55–58]. C-mitosis indicates the chemical inhibition of spindle formation mainly associated with spindle poisoning [59]. Since the plant extracts/fractions did not show C-mitotic effect, it suggests that they could activate spindle formation, although it may not be very significant. Chromosomal fragmentation is an indication of chromosome breaks, and can be a consequence of anaphase/telophase bridges [60,61].The plant extracts/fractions may not only have interfered with the cell cycle but could have also affected chromatid organisation of the DNA replication, causing chromosome breaks as observed in this study. The total chromosomal aberrations increased significantly on exposure to the plant extracts/fractions which indicates clastogenic effect (an effect induced by disruption or breakage of chromosome which may influence chromosomal deletion, addition or rearrangement).The results obtained were in compliance with reports of other research findings of various medicinal plants [62].Binuclear chromosomes were not observed and may have been as a result of cytokine activation [63].

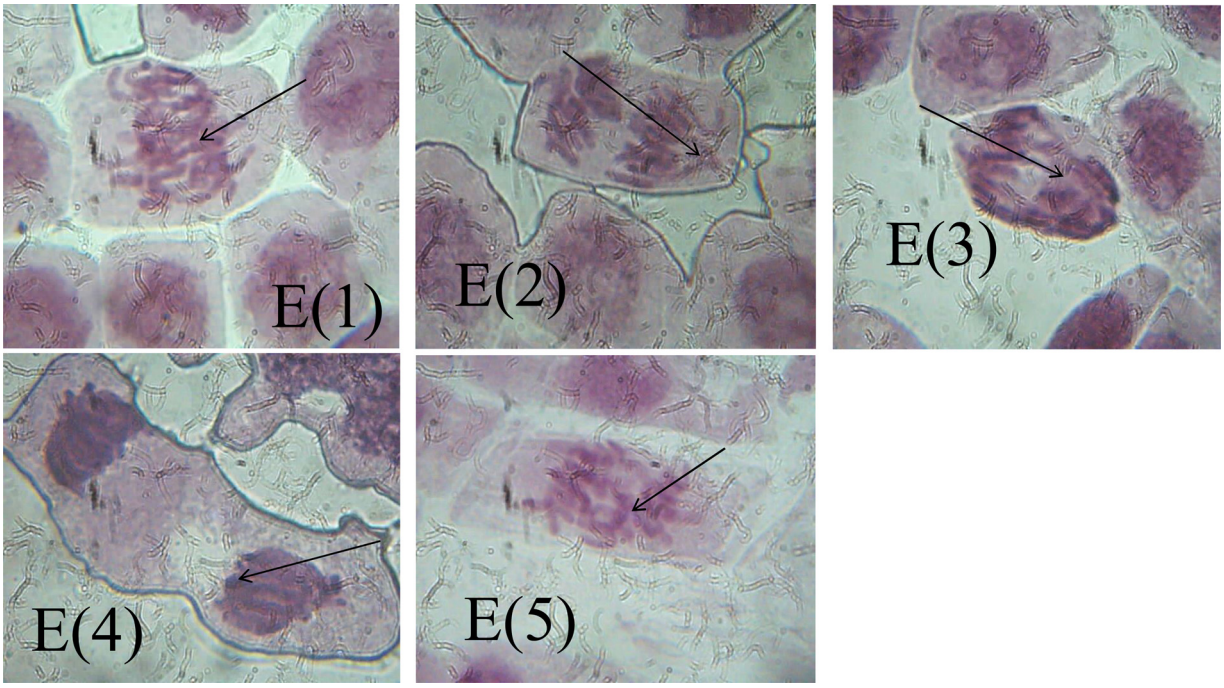

Fig. 5. Photomicrographs showing the effect of methanol extract of *M. oleifera* E(1) Bridged anaphase E(2) Vagrant E(3) Bridged telophase E(4) Sticky E(5) Bridged and fragmentation.

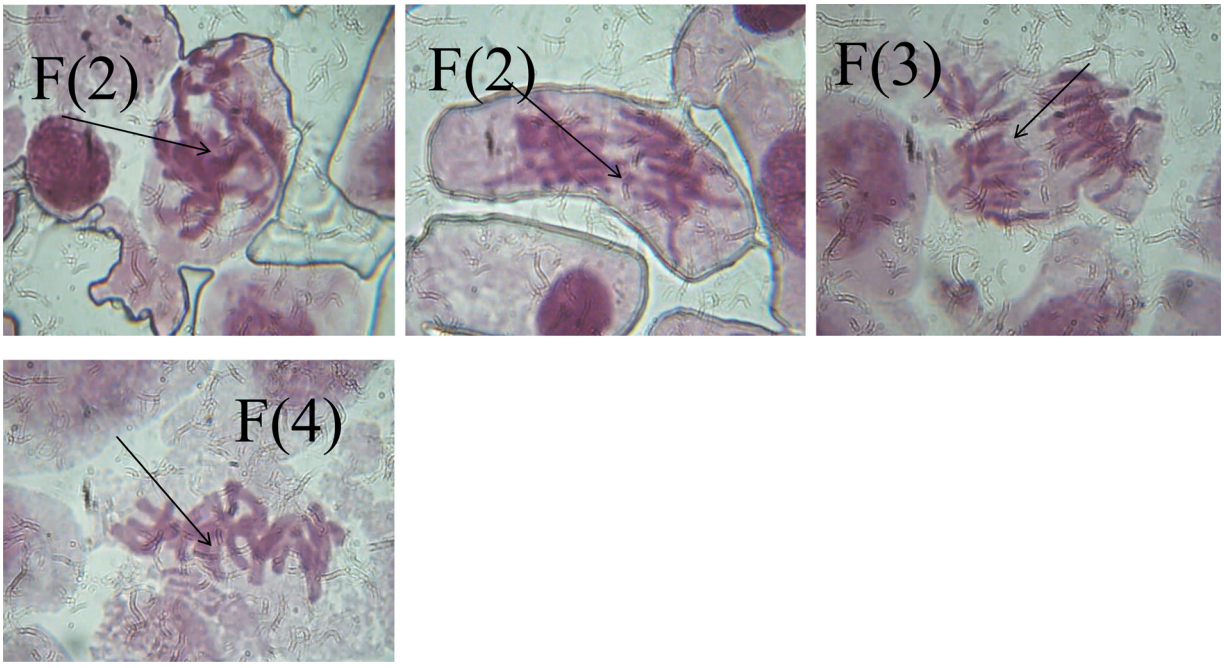

Fig. 6. Photomicrographs showing the effect of ethylacetate fraction of *M..oleifera* F(I) Vagrant F(2) Bridged anaphase F(3) Multipolar F(4)Attached.

5. Conclusion

The general concept of people about herbal products is that they are safe to the human system owing to the fact that they are natural products. The present study has shown that herbal products could be toxic to cells. Scientific results obtained from this study showed that methanol extracts/fractions of *T.bangwensis* and *M.oleifera* exhibited antioxidant activity. However, they showed cytotoxic effect. The cytotoxicity was characterised by reduction in mitotic index, number of dividing cells, inhibition of root tip growth length and induction of various types of chromosomal aberrations in the root tip cells of *A.cepa*. Therefore, the present study demonstrates that at 100 mg/100 mL, cytotoxic effect is significant compared to other concentrations.

Authors comments

All corrections mentioned by the reviewers have been due attended to and other correction I found without reviewers comments

Funding or financial support

Authors state unequivocally that the present study did not receive

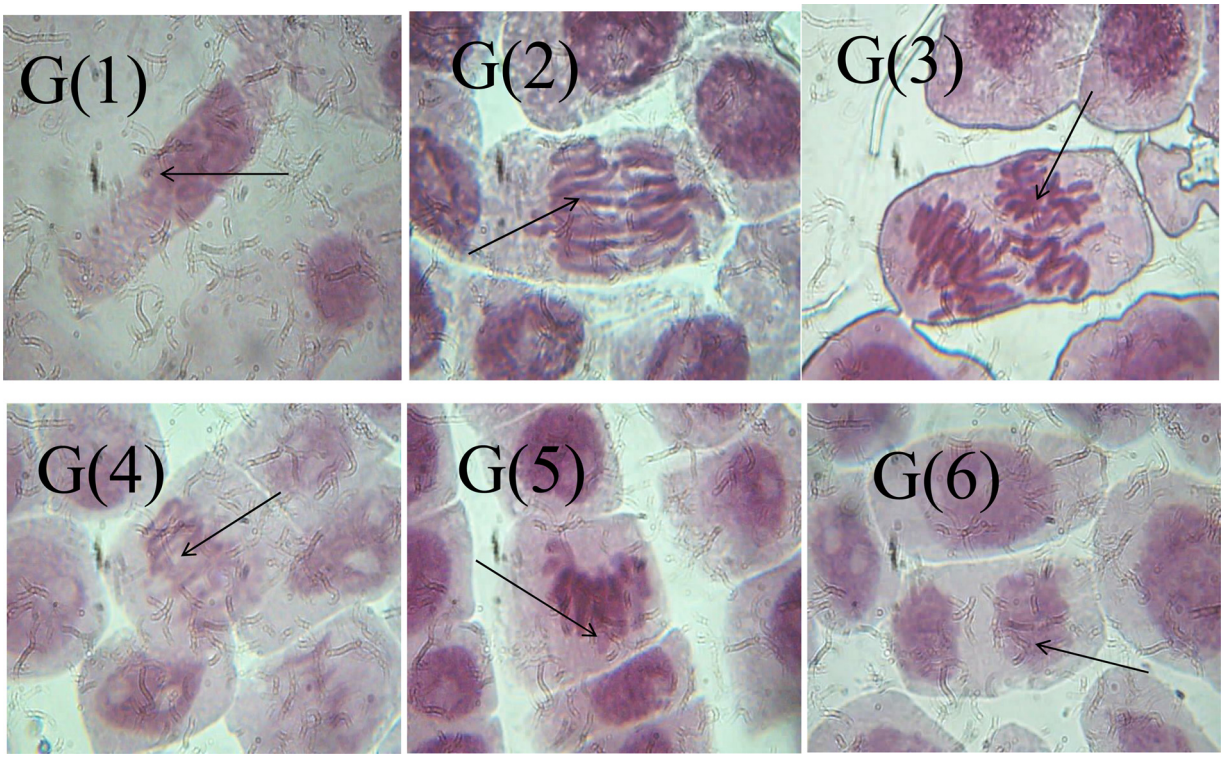

Fig. 7. Photomicrographs showing the effect of acetone fraction of *M. oleifera* G(1) Vagrant G(2) Bridged anaphase G(3) Multipolar G(4) Bridged G(5) Attached G(6) Sticky telophase.

any funding or financial support from any external source be it from institution or corporate bodies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We appreciate the technical efforts of Mr. Francis Obu, Department of Cell Biology and Genetic, University of Lagos and Mrs. Lilian Ekeson, Mrs. Hauwa Yakubu and Miss Gladys Ihegboro, Department of Biochemistry and Forensic Science, Nigeria Police Academy, Wudil, Kano.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.05.001.

References

[1] A.A. Njan, S.O. Olaoye, S.O. Afolabi, B.C. Ejimkonye, A. Soje, O.E. Olorundare, E.O. Iwalewa, Safety effect of fractions from methanolic leaf extract of *Ocimum gratissimum* on reproduction in male wistar rats, Toxicol. Rep. 6 (2019) 496–504.
[2] A.M. Welia, H.S. Al-Saadia, R.S. Al-Fudhailia, A. Hossaina, Z.B. Putitb, M.K. Jasimc, Cytotoxic and antimicrobial potential of different leaves extracts of *R.fRuticosus* used traditionally to treat diabetes, Toxicol. Rep. 7 (2020) 183–187.
[3] S.O. Ogbonnia, F.E. Nkemehule, E.N. Anyika, Evaluation of acute and subchronic toxicity of *Stachytarpheta angustifolia* (Mill) Vahl (Fam. Verbanaceae) extract in animals, Afr. J. Biotechnol. 8 (2009) 1793–1799.
[4] D. Jeya, I. Sheela-Jeeva, M. Ramzan-Shamila, N.C.J. Packia-Lekshimi, J. Raja-Brindha, Asian. J. Plant. Sci. Res. 2 (1) (2012) 41–44.
[5] W.H. Lewis, M.P.F. Elvin, Leaves, Medicinal Botany Plants Affecting Man's Health, John Wiley and Sons, New York, 1997, p. 515.
[6] M. Prabakaran, N. Chandrakalia, A. Panmer-Selvan, Asian. J. Plant. Sci. Res. 1 (2) (2011) 18–25.
[7] T.J. Mosley, Econ. Bot. 48 (4) (2009) 397–412.
[8] M.S. Butler, The role of natural product chemistry in drug discovery, J. Nat. Prod. 67 (2004) 2141–2153.
[9] M.J. Moshi, D.F. Oueno, P.K. Mbabazi, A. Wisheit, The ethnomedicine of the haya people of Bugabo and Kagera Region, north-western Tanzania, J. Ethnobiol. Ethnomed. 5 (1994) 24.
[10] J. Westendoff, Plant toxins, in: H. Marquardt, S.G. Schafer, R.O. McClellan, Welsch (Eds.), Toxicology, 5th Ed., Academic press, California, 1999, pp. 959–1007.
[11] P. Van der Biji Jr., P. Van der Biji, (Sen), Cardiotoxicity of plants in South African, Cardiovasc. J. S. Afr. 23 (9) (2012) 476–477.
[12] R.J. Abdualmijid, C. Sergi, Hepatoxin botanicals: an evidence-based systematic review, J. Pharma. Pharmacet. Sci. 16 (3) (2013) 376–404.
[13] P.O. Osadebe, I.C. Uzochukwu, Chromatographic and anti-motility studies on extracts of *Loranthus micranthus* Linn, J. Pharmaceut. Allied Sci. 3 (2006) 263–268.
[14] R. Grossarth-Maticek, R. Ziegler, Prospective controlled cohort studies on long-term therapy of ovarian cancer patients with mistletoe (*Viscum album*) extracts, Iscador.Arzneimittel. Forschung 57 (2007) 665–678.
[15] G.O. Ihegboro, C.J. Ononamadu, E. Afor, G.D. Odogiyan, Cytotoxic and hepato-curative effects of aqueous extract of *Tapinanthus bangwensis* against paracetamol-induced hepatotoxicity, J. Evidence-Based Integr. Med. 27 (2018) 1–7.
[16] P.O. Osadebe, C.A. Dieke, F.B.C. Okoye, A study of the seasonal variation in the antimicrobial constituents of the leaves of *Loranthus micranthus* sourced from *Percia americana* Res, J. Med. Plants 2 (2008) 48–52.
[17] F. Anwar, M.I. Bhanger, Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan, J. Agric. Food Chem. 51 (2003) 6558–6563.
[18] J.W. Fahey, *Moringa oleifera*: a review of the medicinal evidence for its nutritional, therapeutically and prophylactic properties, Part 1, Tree of Life 1 (2003) 1–5.
[19] S. Faizi, Pharmacological studies on hypotension and spasmolytic activities of pure compounds of *Moringa oleifera*, Phytother. Res. 8 (1994) 87–91.
[20] D.F. Wiwt, E. Taslim, S. Kumiyoshi, F. Sri, Antioxidant activity of *Moringa oleifera* extracts, Indonesian J. Chem. 16 (3) (2016) 297–301.
[21] A. Farahziela, N.M.T. Che, A.M.M. Mohamad, M.A. Sobri, Antioxidant properties of crude extracts, partition extract and fermented medium of *Dendrobium sabin* flower, Evid-based Compl. Alternat. Med. 2017 (2017) 29077219.
[22] J.B. Harbourne, Phytochemical methods, Chapman and Hall Limited, London (1973), pp. 49–188.
[23] C. Chang, M. Yang, H. Wen, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J. Food. Drug. Anal 10 (2002) 178–182.
[24] K. Slinkard, V.L. Singleton, Total phenol analysis: automation and comparison with manual methods, Am. J. Enol.Viticult. 28 (1977) 49–55.
[25] T.P. Van-Burden, W.C. Robinson, Formation of complexes between protein and Tannin acid, J. Agric. Food. Chem. 1 (1981) 77–82.
[26] B.O. Obadoni, P.O. Ochuko, Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta states of Nigeria, Glob.

J. Pure. Appl. Sci 8 (2) (2001) 203–208.
[27] M. Burtis, F. Bucar, Antioxidant activity of *Nigella sativa* essential oil, Phytotherapy. Res. 14 (5) (2000) 323–328.
[28] C.S. Alisi, G.O.C. Onyeze, Nitric oxide scavenging ability of ethylacetate fraction of methanolic leaf extracts of *Chromolaena odorata* (Linn.), Afr. J. Biochem. Res. 2 (2008) 145–150.
[29] G. Fiskesjo, The *Allium cepa* test as a standard in environmental monitoring, Herediteas 102 (1985) 92–112.
[30] A. Vimala, T. Thamizharasi, S.S. Sathish, R. Palani, P. Vijavakanth, Phytochemical studies on selective medicinal plants, Int. J. Res. Eng. Biosci. 1 (2013) 57–62.
[31] G.O. Ihegboro, C.J. Ononamadu, A.T. Owolarafe, E. Afor, I.K. Zaharadeen, Antioxidants in plant extracts may contribute to the modulation of their toxicity: an insight with *Allium cepa* model, NISEB J. 18 (2) (2018) 92–104.
[32] I.E. Josepha, I.F. Jajab, A.H. Boyia, O.M. Olugbengaa, Comparative effects of methanol and oil extracts of *Ocimum gratissimum* on testicular morphology and epididymal sperm reserve of adult male albino rats (Wistar strain), Toxicol. Rep. 6 (2019) 1127–1134.
[33] A. Mayowa, D. Tabajara, D.O. Martins, S. Olaitan, *Ocimum gratissimum L.* leaf flavonoid-rich fraction suppress LPS-induced inflammatory response in RAW 264. 7 macrophages and peritonitis in mice, J. Ethnopharmacol. 204 (2017) 169–178.
[34] K.O. Akinyemi, O. Oladapo, C.E. Okwara, C.C. Ibe, K.A. Fasure, Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity, BMC Compl. Altern. Med. 7 (2005) 1–7.
[35] V. Smaka-Kinel, P. Stegnar, M. Lovka, The evaluation of waste, surface and ground water quality using the *Allium cepa* test procedure, Mutat. Res. 368 (1996) 171–179.
[36] B.B. Babatunde, A.A. Bakare, Genotoxicity screening of waste waters from Agbara industrial Estate, Nigeria evaluated with *Allium cepa* test, Pollut. Res. 25 (2) (2006) 227–234.
[37] A. Akinboro, A.A. Bakare, Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn, J. Ethnopharmacol. 112 (2007) 470–475.
[38] A. Oloyede, J. Okpuzor, O. Omidiji, Cytological and toxicological properties of a decoction used for managing tumors in South-Western Nigeria, Pak. J. Biol. Sci. 12 (4) (2009) 383–387.
[39] A.E. Adegbite, E.B. Sanyaolu, Cytotoxicity testing of aqueous extract of bitter leaf using the *Allium cepa* chromosome aberration assay, Sci. Res. Essay 4 (11) (2009) 1311–1314.
[40] D.I. Olorunfemi, G.E. Okoloko, A.A. Bakare, A. Akinboro, Cytotoxic and genotoxic effects of cassava effluents using the *Allium cepa* assay, Res. J. Mutagen 1 (2011) 1–9.
[41] E. Rojas, L.A. Herrera, M. Sordo, Mitotic index and cell proliferation kinetics for identification of antineoplastic activity, Anti-Cancer Drug 4 (6) (1993) 637–640.
[42] K.Y. Ping, I. Darah, U.K. Yusuf, S. Sasidharan, Genotoxicity of *Euphorbia hirta* in *Allium cepa* assay, IPCBE 39 (2012) 222–225.
[43] M.M. Hoshina, Avaliacao da possivel contaminacao das aguas do Ribeirao claro-municipio de Rio claro, pertencente a bacia do rio corumbatai por meio de testes de mutagenicidade em A*llium cepa* Trabalho de conclusao (Bacharel e Licenciatura-cencias Biologicas, universidade Estadual Paulista, Rio. Claro. Sp. (2002) 52.
[44] D.M. Leme, H. Marin-Morales, *Allium cepa* in environmental monitoring: a review on its application, Mutat. Res. 682 (2009) 71–81.
[45] J. Vant-Hooff, The action of IAA and kinetic on the mitotic cycle of proliferative and stationary phase existed root meristem, Exp. J. Cell Res. 51 (1968) 167–176.
[46] E. Bruneri, Synthesis of DNA and mitosis in relation to cell differentiation in the roots of *Vicia faba* and *Lactura sativa*, Cytologia 36 (1971) 229–247.
[47] M. Metin, B. Burun, Effects of the high doses of *Urgine maritima* (Linn) baker extracts on chromosomes, Cytologia 63 (4) (2010) 367–375.
[48] R. Sudhakar, K.N. Ninge-Gowda, G. Venn, Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*, Cytologia 66 (3) (2001) 235–239.
[49] E. Schulze, S. Krischer, Microtubules dynamics in interphase, Cellular J. Cell. Biol. 102 (1996) 1020–1021.
[50] Y. Sheng, C. Brygelsson, K.W. Pero, Enhanced DNA repair, immune function and reduced toxicity of C-MED-100, a novel aqueous extract from *Uncaria tomentosa*, J. Ethnopharmacol. 69 (20) (2002) 115–126.
[51] M. Kuras, J. Augustynowicz, E. Sliwinska, K. Pilarski, K. Ilasz, T. Tykarska, A. Zobel, K. Gulewicz, Changes in chromosome structure, mitotic activity and Nuclear DNA content from cells of *Allium cepa* test induced by bark water extract of *Uncaria tomentosa* (wild) D.C, J. Ethnopharmacol. 107 (2) (2007) 211–221.
[52] F. Khakdan, K. Piri, Cytotoxic and genotoxic effects of aqueous root extract of *Arctium lappa* on *Allium cepa* root tip cells, Int. J. Agron. Plant. Prod. 3 (2) (2012) 630–637.
[53] J. Stephen, Cytological causes of spontaneous fruit abortion in *Haemanthus katherinae* Baker, Cytologia 44 (1996) 805–812.
[54] V.C. Mercykutty, J. Stephen, Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test, Cytologia 45 (4) (1980) 769–777.
[55] E. Rencuzogullari, H.B. Ila, A. Kayralodiz, M. Topaktas, Chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated with sodium metabisulfite, a food preservative, Mutat. Res. Mutagen. Relat. Subj. 490 (2) (2001) 107–112.
[56] A.A. EL-Ghamery, M. EL-Kholy, M.A.A. EL-Yousser, Evaluation of cytological effects of Zn^{2+} in relation to germination and root growth of *Nigella sativa* Linn. and *Triticum aestivum* Linn, Mutat. Res. 537 (2003) 29–41.
[57] A.N. Gomurgen, Cytological effect of the potassium metasulphite and potassium nitrate food preservative on root tips of. *Allium cepa*, Cytologia 70 (2) (2005) 119–128.
[58] S. Turkoglu, Genotoxicity of five food preservatives tested on root tips of *Allium cepa* Linn, Mutat. Res./Genet.Toxicol. Environ. Mutagenesis 26 (1-2) (2007) 4–14.
[59] S.A. Shahin, K.H.H. EL-Amoodi, Induction of numerical chromosomal aberrations during DNA synthesis using the fungicides nimrod and rubigan-4 in root tips of *Vicia faba* Linn, Mutat. Res. 261 (3) (1996) 69–176.
[60] A. Sharma, S. Sen, Chromosome Botany. Enfield, NH, USA, 2002, p. 45.
[61] R.J. Singh, Plant Cytogenetics. Boca Raton, Fla, CRC Press, USA, 2003, p. 78.
[62] C.J. Bidau, A.G. Amat, M. Yajia, D.A. Marti, A.G. Riglos, A. Silvestroni, Evaluation of the genotoxicity of aqueous extracts of Ilex paraguariensis St. Hil.(Aquafoliaceae) using the *Allium cepa* test, Cytologia 69 (2) (2003) 109–117.
[63] G.C. Kaushik, Cytological effects of *Lantana camara* Linn leaves extract on *Vicia faba* root tip cells, Life Sci. Adv. Plant Physiol. 9 (1996) 159–164.