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Genotoxicity and carcinogenicity of *Frosskaolea tenacissima* (Linn) leave extract used in traditional medicine in Saudi Arabia

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The present work aimed to evaluate the potential carcinogenic, cytotoxic, and genotoxic effects of methanolic extract leaves of *Frosskaolea tenacissima* L. found in Makkah region, Saudi Arabia. The Mitotic Index (MI) was determined for cytotoxicity studies, and micronuclei (MN), chromosomal aberrations (CA) were counted, and a molecular marker (ISSR-PCR assay) was performed to study the genotoxicity, and then the DNA amount and fragments in comet cells were figured for the carcinogenicity of each 0.27, 0.55, 1.10, and 1.37 mg/ml concentration of the crude extracts of *F. tenacissima* using the *Allium cepa In Vivo* bioassay. The results showed that *F. tenacissima* methanolic extract exerted significant cytotoxic and genotoxic effects on *A. cepa* cells with significant values for concentrations from 0.55 mg/ml, 1.10 mg/ml, and 1.37 mg/ml. It was found that the low growth of the meristematic cells of *A. cepa* roots, and increased significantly the mitotic index compared with the control, and also, the high micronucleus and chromosomal aberrations count showed the presence of genotoxicity/ or cytotoxicity, as well as a low number of DNA damage in comet cells, which indicated dose-dependent carcinogenic potential effects of this plant. These results suggest *F. tenacissima* is expected to have the same effects on human cells. So, these mutagenic and genotoxic effects on human cells should be investigated in further studies.

Keywords: Cytotoxicity, Genotoxicity, Carcinogenicity, *Frosskaolea tenacissima, Allium cepa*.

INTRODUCTION

Medicinal plants are used for their therapeutic properties in traditional and modern medicine (Yuan *et al.* 2016; Qari *et al.* 2021a). Medicinal plants are rich in secondary compounds which are the remediation and prevention of diseases (Fan *et al.* 2023). According to WHO, (2002), up to 80% of the world's population especially in developing countries use traditional drugs including medicinal herbs to meet their primary healthcare needs. In Saudi Arabia, a large number of traditional plants are still in use as a source of herbal therapies (Al-Yahya *et al.* 1990; Qari, 2008).

Forsskaolea tenacissima L. belongs to the genus *Forsskaolea*, and is synonyms: *Caidbeja adhaerens* (Forssk) and *Forsskaolea cossoniana* (Webb). It is represented in North Africa, extending to Southeast Spain. Sudan, Ethiopia. Palestine, Saudi Arabia, Iran and India (Boulos, 1999). This plant is very common in Saudi Arabia mainly in the south-west (Egazy *et al.* 1998), and in the western region (Qari *et al.* 2021b), *F. tenacissima* is rich in active compounds like sterols, triterpenes and flavonoids, tannins, phenolics and forsskamide (Qaisar *et al.* 2008; Assaf *et al.* 2020). Moreover, it is used as an anti-inflammatory, antispasmodic, antidiabetic, and antipyretic (Shah *et al.* 2010), antioxidant (Alali *et al.* 2007; Attia *et al.* 2021), hepatoprotective (Assaf *et al.* 2017), antinociceptive, antipyretic (Sher *et al.* 2017), antiviral and antibacterial activities (Assaf *et al.* 2015). In Saudi Arabi, it`s largely used in rural and urban areas for the treatment of many diseases such as wound healing, and removal of bile stone from gall bladder also has antioxidant potential and larvicidal activity (Sher *et al.* 2017; Attia *et al.* 2021).

Although the significant medicinal benefits some of the plants provide, some constituents of medicinal plants have been shown to be potentially toxic, genotoxic and carcinogenic activities (Moody *et al.* 1999). However, there are no reports on the carcinogenic, cytotoxic, and genotoxic properties of this plant in literature. As part of an ongoing study on genetic safety evaluation of herbal extracts commonly used in Saudi Arabia, this study was conducted to evaluate the potential genotoxicity and carcinogenicity of *F. tenacissima* methanolic leaf extract found in Makkah region, Saudi Arabia using cytogenetic and molecular assays on *Allium cepa* in the perspective of assessing the genetic safety of using medicinal plants in traditional medicine.

MATERIALS AND METHODS

Sample collection

The leaves of *F. tenacissima*, samples belongs to the family Urticaceae were collected on 2020- 2021 from Makkah region, located in Saudi Arabia at latitude 21° 37' 29.99" N and longitude 40° 26' 50.82" E (Figure 1). The samples were authenticated by a taxonomist in the Umm Al-Qura University, Saudi Arabia.

Figure 1: Sample collection site, Makkah region, Saudi Arabia, (google map, https://2u.pw/l3zwkWg).

Preparation of Plant Extracts

According to the study of Ping *et al. (*2012) with modifications in methodology, 100g of plant-dried leaves of *F. tenacissima* were ground with a grinder and soaked in a glass bottle with 400 ml of methanol for 72 hr., shaken 3-4 times per day, and then filtered through a clean muslin cloth followed by double filtration. Whatman No. 1, after three days, used a rotary evaporator under low pressure and vacuum to evaporate methanol from extraction at 50°C, and then poured the extract into a petri dish and placed it in an oven at 55°C to dry all the methanol from the crude extraction. The Petri dish was covered with Parafilm and stored in a dark cabinet until use. Next, 10 mg/mL of the crude methanol extract was dissolved in 100 mL of distilled water to prepare stock, which was then diluted to make different concentrations of the methanolic extract.

Determination of Root Growth Inhibition and EC50

Forty seeds of *A. cepa* (Sunshine Seed Co., USA) were placed on filter paper in each of the ten Petri dishes for each concentration of methanolic extract. Before germination, the seeds of *A. cepa* were washed twice with distilled water, then treated with six concentrations of methanolic crude extracts 0.5, 1, 1,5, 2, 2.5 and 3 mg/ml, and distilled water was used as a negative control. The seeds were grown for five days in the dark at 25°C. The length of the roots of germinated seeds was measured. The root growth percentage inhibition in relation to the control for each extract was determined, followed by determining the effective concentration that decreased root growth to 50% when compared to the negative control, as described by Dragoeva *et al.* (2015).

Allium cepa Bioassay

For cytogenetic and molecular experiments determining EC50 of *F. tenacissima*, the forty seeds of *A. cepa* were washed twice with distilled water for each petri dish and placed on sterile filter paper placed in the dark at 25°C to grow, then watered at 3 ml after five days, treated 2 ml with different concentrations of the crude extracts 0.27, 0.55, 1.10 and 1.37 f mg/ml for 24, 48, and 72hrs. Another set of plants was placed in ethyl methane sulfonate (1% EMS) as positive control while the water was a negative control. The root tips (1-3 cm) from each seedling were harvested, and fixed in Carnoy's fixative (1:3 acetic acid: alcohol) for 24 hr. It then proceeded to slide preparation or stored in 70% alcohol **(**Fiskesjö, 1985; Qari, 2016)

Slides Preparation

The preparation of slides was carried out according to Sharma and Sharma, (1980). After pre-treatment, the root tips were washed a few times with distilled water. They were hydrolyzed with 1 N HCl at 60°C for 5-8 minutes. After hydrolysis, the roots were washed in 70% ethanol twice for an hour and then placed for 1-6 hrs. in the acetocarmine. Then, about 2–3 mm of the root tips were cut and placed on the slide. A small drop of acetocarmine stain was dropped on the root tip and left for 2 min. The root tip was then crushed with an inoculation needle and the coverslip was carefully lowered to avoid air bubbles and the sides of the slides were sealed with clear a rubber solution. The slides were examined with the objective lens 40X and the eyepiece 20X, so approximately 100 cells were examined on one slide in five different areas, and accordingly, the total number of cells that were examined in one treatment for one period was 1000 cells (Qari *et al.* 2021).

Chromosomal Aberrations and Mitotic Index

The numbers of divided and non-divided treated cells of *A. cepa*, the different phases of mitosis, chromosomal abnormalities, and mitotic abnormalities were counted and photographed. The most common chromosomal aberrations were identified, such as bridges, chromosomal fragments, sticky chromosomes, and micronuclei. Also calculated is the mitotic index, which is divided by the number of cells in mitosis by the total number of cells multiplied by 100, according to Sidorki, (1984).

DNA Extraction

After one week of growth, *A. cepa* roots were washed several times with sterile ddH2O for ten minutes, then dried and ground in liquid nitrogen, until they became a

powder, and the CTAB technique was followed to extract *A. cepa* DNA according to Allen *et al.* (2006) with some modifications. The relative purity of the DNA samples was carried out on UV Spectrophotometer. Also, the purity of DNA for all samples was confirmed with a nano drop spectrophotometer, according to the nano drop manual from Thermo Fisher Scientific. Agarose gel (1.2 %) electrophoresis was conducted to visually assess DNA quality. For PCR amplification, each sample was diluted to 50 ng/µl concentration and samples were stored at -20°C.

ISSR-PCR Marker

To assess the genotoxic potential of *F. tenacissima* utilizing molecular markers such as ISSR-PCR for polymorphism. After extracting DNA from the *A. cepa* root, use 4 ISSR primers (ISSR418, ISSR-HB12, ISSR-UBS811, and ISSR-MAO), Macrogen, South Korea, and optimize the annealing temperature for each primer according to Wolfe, (2005) and Sudha *et al.* (2019). Four samples for methanolic extraction were subjected to ISSR-PCR markers, and one sample as a negative control. For one PCR reaction, 25µl volume was used, containing 10µl of 2x master mix, 2µl of 10µM primer, 2µl of DNA template, and 6µl of nuclease-free water. Also, the master mix contains dNTP, MgCl2, Taq polymerase, and TBE buffer. The PCR thermal cycle was started with an initial denaturation at 94 oC for 5 minutes, then denaturation at 94 oC for 30 seconds, annealing at 42 oC for 45 seconds, and extension for 1.5 minutes at 72 oC. This cycle was repeated for 35 cycles, the PCR product was left for 7 minutes at 72°C for final extension, and the last step was held at 4°C (Wiesner and Wiesnerová, 2003).

Carcinogenesis Assessment

Preparation Sample for Comet Assay

The root tips (about 5 mm) from control and treated seedlings of *A. cepa* exposed to EC25 (0.55mg/ml) and EC75 (1.37mg/ml) of crud extract of *F. tenacissima* for 24, 48, and 72 hrs. were cut and immediately chopped with 500µl precooled Galbraith's buffer (45 mM MgCl26H2O, 30 mM C6H5Na3O72H2O, 20 mM MOPS, 0.1% TritonX-100, pH 7.0). And then the isolated root nuclei will filter through a 600-mesh sieve to obtain the nuclear suspension.

Comet assay (Single Cell Gel Electrophoresis)

To evaluate the DNA damage (carcinogenicity) score using a comet assay. This technique was conducted using the Comet assay Kit along with manual preparation for standard microscope slides that were pre-coated with the first layer of 1% normal melting point agarose (NMPA), thoroughly dried overnight at room temperature, and kept dry in slide boxes. After the slides were incubated for 20 min to allow the DNA in the nucleus to unwind and then electrophoresed for 30 min at 4°C at 0.72 V cm^{1} (19 V, 300 mA). After electrophoresis, the slides will be soaked in absolute ethyl alcohol for five minutes before being rinsed twice with 400 mM Tris-HCl solution (pH 7.5) to neutralize the results. The dry slides were stained for 5 mins with SYBR Gold that had been diluted (1:10000, Life Technologies, USA, S-11494) before being rinsed with ice-cold deionized water to remove the excess dye. Fluorescence microscopy was used to investigate the comet cells (Nikon ECLIPSE50i, Japan). CASP software (v 0.2) was used to measure and analysis 20 randomly selected comet images for each plate (Końca *et al.* 2003). As a measure of DNA damage, the "Olive Tail Moment" (OTM) was derived (Kumaravel and Jha, 2006).

Data Analysis

Several statistical methods were used to present and analyze the cytogenetic results, the mitotic index, the ratio of mitotic phases, and the percentage of each chromosomal aberration were generated, according to Sidorki, (1984). The gel electrophoresis image was analyzed using Quantity One version 4.6.2 by comparing results with the control and also to find phylogenetic dendrograms and polymorphisms between different concentrations. The statistically significant difference, calculated by SPSS Statistics version 28.0.1.1, also used one-way various analyses (ANOVA).

RESULTS

Determination of EC50

Figure 2 shows the results of the EC50 for the crud extract of varying concentrations of *F. tenacissima* leaves against the *A. cepa* seeds. The results showed that the methanolic extract also caused inhibition in *A. cepa* root length at high concentrations (0.5 1, 1.5, 2, and 2.5 mg/ml), which resulted in a 50% inhibition of the length of the *A. cepa* root (EC50) for methanolic extract at 1.1 mg/ml.

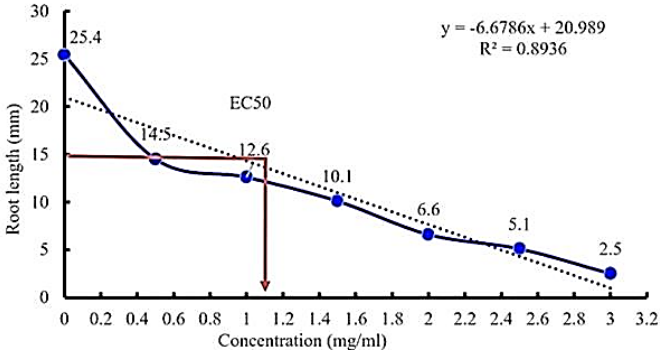

Figure 2: Linear regression for the percentage of root length of *A. cepa* after treatment with different concentrations of the methanolic extract of *F. tenacissima* leaves, and determination EC50.

Growth inhibition of Meristematic Cells in *A. cepa* Roots

Figure 3 shows the results of the effects of the crude extract of *F. tenacissima* on the root growth of *A. cepa*. This showed an inverse relationship between the concentrations of *F. tenacissima* extract and the inhibition of cell division in the roots of *A. cepa*, as well as a positive relationship between exposure periods and the decrease in the rate of root growth inhibition. Inhibition of root growth was concentration dependent and statistically significant ($P < 0.05$) at EC75 (7.5 mg/ml) of concentration was 21,87%, 30,68%, and 32.6%, respectively, compared to the negative control, and at same of concentration was 39.54 %, 46.22 %, and 51.07 % compared to the positive control (EMS). Inhibition of root growth which suggests high toxicity of the plant.

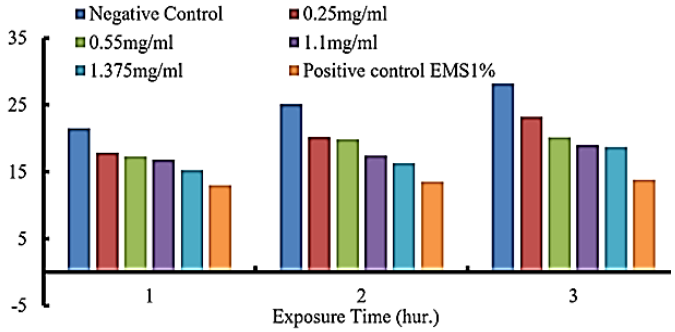

Figure 3: Root length of *A. cepa* after treatment with different concentrations of methanolic leaves extraction of *F. tenacissima* for different exposure times.

The effects of treating the meristem cells of the *A. cepa* roots with different concentrations of the methanolic extract of *F. tenacissima* leaves on the division phases were presented in Figure 4, A and B. the mentioned findings in Figures 4, 5, the meristem root cells' mitotic index decreased linearly as the concentration of the methanolic extract of *F. tenacissima* leaves and the exposure time increased with a correlation coefficient (r^2) of 0.9937. and 0.9937, respectively.

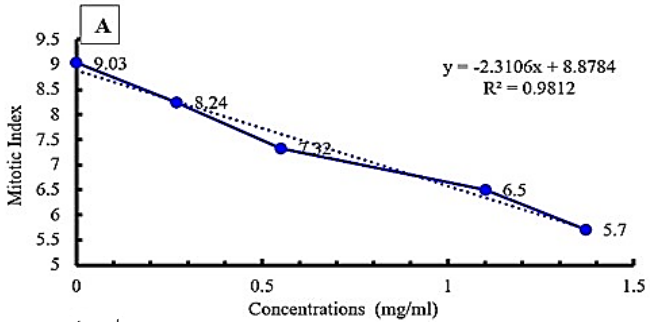

Figure 4. (A): Relationship between the mitotic index of *A. cepa* and methanolic extract of *F. tenacissima* at different concentrations (mg/ml).

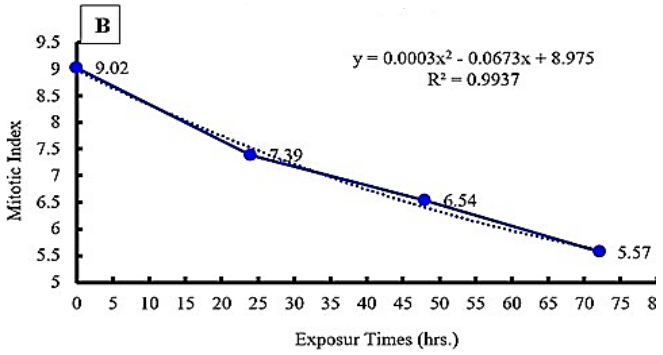

Figure 4. (B): Relationship between the mitotic index of *A. cepa* and methanolic extract of *F. tenacissima* at different periods of time (hrs.) compared with the control.

Genotoxic Effects of Methanolic Extract of *F. tenacissima* Leaves

The results in Figure 5 (A and B) shows that the frequency of metagenesis in *A. cepa* root meristem cells after treatment with different concentrations (0.27, 0.55, 1.10, and 1.37 mg/ml) of methanolic extract of *F. tenacissima* leaves and different times (24, 48, and 72 hrs.) which was 0.219, 0.268, 0.410, and 0.645, respectively, for various concentration 0.333, 0.362,and 0.453, respectively, for different times when compared to the control (0.022). It reveals the strength of the direct relationship between mutation frequency and an increase in the methanolic extract concentrations and time exposure of *F. tenacissima* with a correlation coefficient (r^2) of 0.9392 and 0.829, respectively. The data indicate that the frequency of mutation increases with the increase in the concentration of the methanolic extract (of *F. tenacissima* leaves and with the increase in exposure periods.

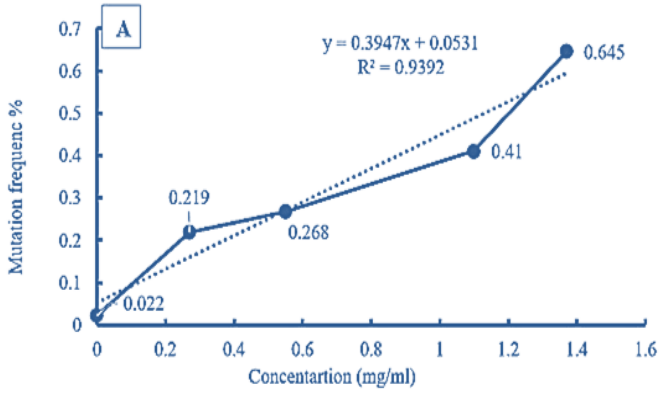

Figure 5 (A): Mutation frequency average against different methanolic concentrations of *F. tenacissima*.

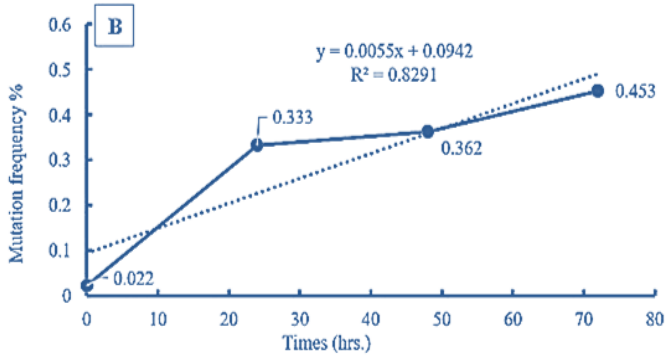

Figure 5 (B): Mutation frequency average against treatment duration times of methanolic concentration of *F. tenacissima*

Abnormalities Formed with Methanolic Extract

The percentage and types of mitotic chromosomal aberrations that were conducted by treatment with a methanolic leave extract of *F. tenacissima* is shown in Figure 6. were as follows: vagrant, fragments, bridge, c-metaphase, disturbance, sickness, and pyknosis. Also, the most abnormalities observed in mitotic root cells of *A. cepa* were micronuclei at the highest concentration (7.5 mg/ml), which were 8.69%, 15.51%, and 30.61% for treatment times 24, 48, and 72hrs., respectively. then vagrant chromosomes, followed by fragments, bridges, and stickiness. Other chromosomal abnormalities were noted in low frequency as c- metaphase and chromosomal disturbances at all concentrations and exposure times. Moreover, pyknosis was recorded as a high rate of death cells (pyknosis) in most treatments, especially during the 72-hr. exposure period for all concentrations used as shown in Figure 6.

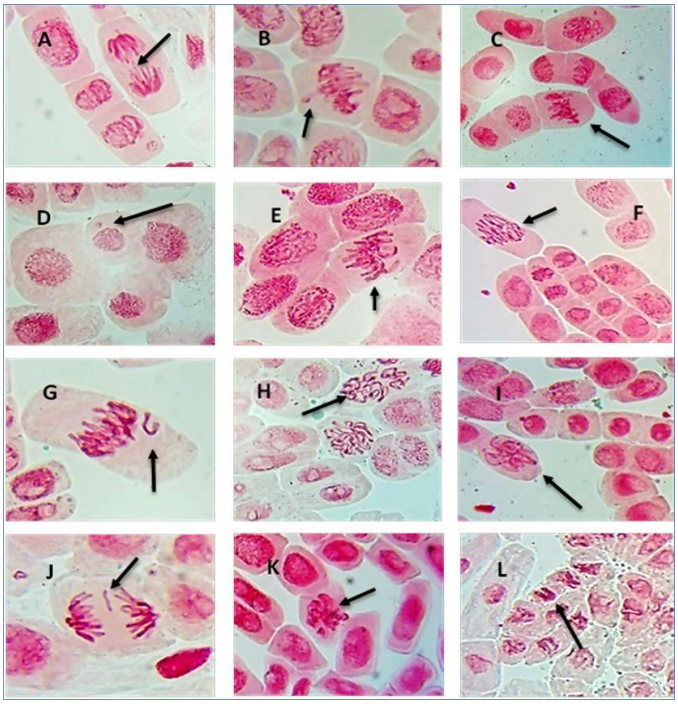

Figure 6: Different types of *A. cepa* meristematic cells showed chromosomal aberration after being treated for 24,48,and 72 hrs. with different concentrations of methanolic extract of *F. tenacissima* leaves. Using an optical microscope on 40X, arrows indicate the abnormalities: (A) bridge; (B) vagrant chromosome; (C) C-metaphase (1.37 mg/ml for 72 hr.); (D) micronucleus; (E,F) C-metaphase (1.10 mg/ml for 48 and 72 hrs.); (G) vagrant chromosome; (H,I) disturbance; (J) chromosome fragments; (K) pyknosis cell in prophase (1.37 mg/ml for 72 hr.); (L) pyknosis cell in telophase (1.37 mg/ml for 48 hr.)

Investigation on Molecular Level

The molecular tests were carried out on the treatment with the concentrations of *F. tenacissima* methanolic extract for the longest exposure time (72hr.) only.

DNA Extraction and Purity Test

After the extraction of DNA by the CTAB method, the purity and size of DNA were determined at the absorbance of wavelengths 260/280 to be around 1.80 ± 0.08 Figure 7.

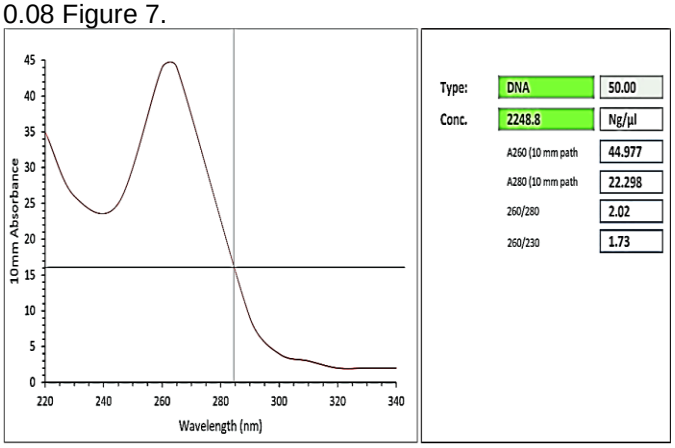

Figure 7: Determining the concentration and purity of DNA samples isolated from different *A. cepa* roots treated with *F. tenacissima* extracts: an example of reading the results from the nanodrop device (i.e., 6 mg/ml for 72 hr.).

ISSR Analysis Results Treated by Methanolic Extraction

Data is presented in Figure 8. A, B, C, and D found that the four primers (ISSR418, ISSR-HB12, ISSR-UBS811, and ISSR-MAO) gave a total of 1-9 genetic bands ranging from 790 to 1200 bp through one primer with each of the different treatments. Different polymorphic bands and weights of genotypes separated from each treatment compared to the control treatment were detected at 0.27, 0.55, 1.10, and 1.37 mg/ml of *F. tenacissima* for 72 hr. The value of the polymorphic rate for the four primers was 100%, 33.3%, 50%, and 44.4% respectively. In all cases, polymorphisms were due to the loss and/or gain of amplified bands in the treated samples compared with the control. Also, Figure 9. A, B, C, and D showed the phylogenetic dendrogram of four

Primers as a marker to show the relationship between treatments of different concentrations of *F. tenacissima* methanolic extract compared to control using UPGMA analysis. The result showed that there are four clades with a low genetic variance of all primers within different distance ranges of about 10–50, 3.7-34.7, 0.00 - 21.0, and 2.1–25.3 respectively, here convergence appears in the evolutionary relations between the control and the treatments based on the concentration, as the high or low-concentration clades differed for different primers having increased or decreased distance from the control clade

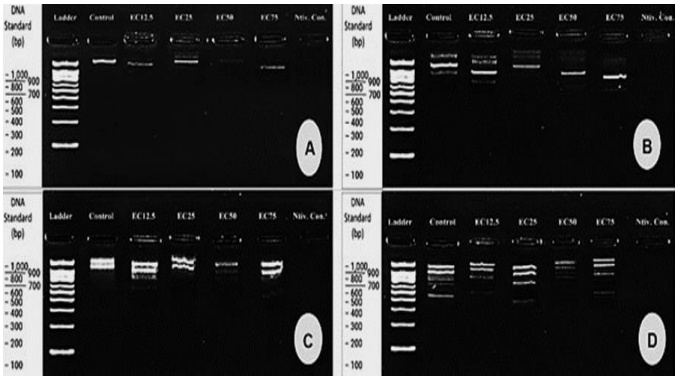

Figure 8: PCR products of ISSR418 (A), ISSR-HB12 (B), ISSR-UBS811 (C), and ISSR-MAO (D) marker amplified with DNA treated with different concentrations of methanolic *F. tenacissima* extract compared to control for 72hr.

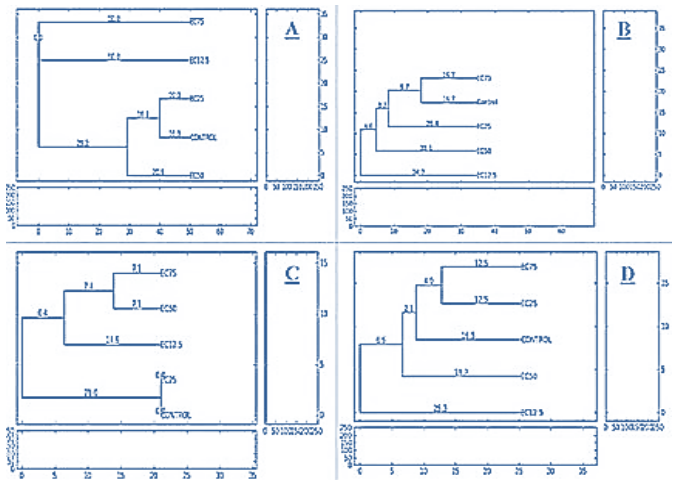

Figure 9: Phylogenetic dendrogram used ISSR418 (A), ISSR-HB12 (B), ISSR-UBS811 (C), and ISSR-MAO (D) as a markers to show relationship after treated with deferent methanolic concentrations of *F. tenacissima* extract compared to control used UPGMA analysis.

Carcinogenicity Investigation by Comet Assay

Data in Figure 10 showed that the comet cells were observed in treatments with large concentrations of *F. tenacissima* for methanolic extract. The highest DNA damage was observed in the EC75 treatment, whereas EC12.5 treatment caused the least comet (%), followed by EC25 and EC50 treatments, and EC50 treatment exhibited the highest intensity of comet detachment from the cell nucleus, while other treatments has moderate comet detachment intensity as compared to the control and normal cells, which is marked by the arrows in Figure 10.

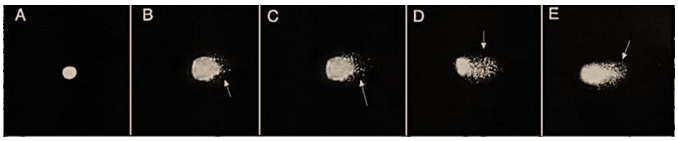

Figure 10: Comet assay images of *A. cepa* meristematic cells treated with methanolic extract of *F. tenacissima* after exposure for 72 hr. two concentrations for each extraction: (A) normal cells that didn't show damage; (B) low levels of DNA damage appear after exposure to 1.10 mg/ml; (C) moderate DNA damage with 6 mg/ml; (D) highly single-strand DNA fragmentation with 1.37 mg/ml; (E) severe DNA damage and long comet tail with 7.5 mg/ml.

DISCUSSION

The potential cytotoxic, genotoxic, and carcinogenic effects of the methanolic extract of *F. tenacissima* on *Allium cepa* root cells were evaluated. The results of the EC50 determination indicated that the higher concentration of methanolic extract (3 mg/ml) led to a decrease of 89.42% compared to the experiment. This is an indication that the extract of *F. tenacissima* may influence the growth of the seed embryo by damaging the mechanism of mitotic division or duplicating the DNA and thus stopping growth, or it may affect the three-nucleated endosperm cells that are loaded with the primary food of the embryo, so the fetus suffers from malnutrition and dies (Dragoeva *et al.* 2015).

The results showed that all the used concentrations of *F. tenacissima* leaf extract caused a significant decrease in the mitotic index compared to the control at all different exposure periods with an inverse function, and this may be due to: one of the components of the plant extract may bind to the terminal PO4 groups (Hartono *et al.* 2012; Suhartono *et al.* 2012) or OH groups (Oulahal and Degraeve, 2022) in the DNA, forming a chelate complex that impedes DNA replication in the cell division cycle, or due to a defect in the biosynthesis of DNA, RNA, or protein (Greenwell and Rahman, 2015). Also, the emergence of some mitotic chromosomal imbalances like metaphase colchicine would disability a path of cell division (Dhooghe *et al.* 2011). Treatment with *F. tenacissima* extract with all different concentrations at all different exposure periods decreased the percentage of the normal mitotic phase and significantly increased the disordered mitotic phase compared to the control treatment this led to a high rate of mutational recurrence, so, this extract of plants one of

the causes of cytotoxic (Lubini *et al.* 2008).

It also showed a significant increase in the ratio of micronuclei and chromosomal abnormalities which increased with increasing concentration and treatment period with *F. tenacissima* extract compared to the control, the micronuclei results in a change in the structure and function of the DNA (Feretti *et al.* 2007; Sommer *et al.* 2020). The extract appears to have an effect like that of colchicine on spindle fibers (Firbas and Amon, 2014).

F. tenacissima leaf extract may act as an anti-spindle fiber by inhibiting the formation of tubulin (kossi, 2017). This phenomenon of collective death of meristematic root cells was recorded in the *A. cepa* plant after treating it with different concentrations of methanolic extract of *F. tenacissima* leaves because the pyknosis of cells increased with increasing concentration of the extract, it maximum reached at a concentration of 1.10 mg/ml after a 24 hr. exposure period, this may be the result of the direct impact of the extract on the ionic medium and functional groups of DNA, and, with a decomposition occurrence for DNA (Ahmed *et al.* 2019; Attia *et al.* 2021). The result indicates that there are one or more components in the *F. tenacissima* plant extract that are able to break the DNA strands in a specific way, and it is believed that this fracture occurs in one of the two DNA strands in exchange, generating chromatid voids as a result of the deletion of one or more nucleotides and this number of fractures and micronuclei appear.

This hypothesis can be supported by the ISSR-PCR results in the previous, which showed great genetic variation in the numbers and weights of bands, which may be the result of this intrusion that would change the structure of reading the frameshift (Fernandes and Vargas, 2003). ISSR-PCR results for the effect of each of the four selected primers (ISSR418, ISSR-HB12, ISSR-UBS811, and ISSR-MAO) were shown for different treatments there was a variation in the polymorphism ratio and weights of genotypes separated from each treatment compared to the control treatment, and this indicates that the extracts of *F. tenacissima* leaves, methanolic, are able to genetic mutations.

After tracking the results of the *A. cepa* test, a significant decrease of the mitotic index on meristem root tips was observed after treating them with different concentrations of the methanolic extract with concentrations (0.27, 0.55, 1.10, and 1.37) mg/ml and different time 24, 48, and 72hrs. The results also recorded an increase in the ratio of cells in the interphase at the expense of the prophase of the division of the stages, while the cells that exceeded the interphase and entered the prophase often stopped at the anaphase, forming colchicine the metaphase. this result is similar to that of Ansah and Gooderham, (2002), Also, the most common phenomenon among cells was pyknosis which was previously registered in the study of Horvitz *et al.* (1994) while exposing the meristem cells to the roots of *A. cepa* and the *Ricinus communis* plant extract.

Several studies also showed the ability of *F. tenacissima* to kill and cause toxic effects on cells, as is the case in Ahmed *et al.* (2019); Assaf *et al.* (2019). This decrease in the amounts of DNA in the *A. cepa* test could also result from deletion mutations that may have occurred due to the ability of a substance or more in the extract on the digestion of DNA (similar to the mechanism of nuclear digester enzymes), especially since there is a direct function between the concentration and the decrease in the amount of DNA on the one hand, and between the exposure period and the amount of DNA on the other hand (Rahimmalek *et al.* 2009).

CONCLUSIONS

A common perception about the use of medicinal plants is that they are harmless to humans due to their natural production. However, results of this study have shown by using molecular and cytogenetic tests that high concentrations of methanol extract of *F. tenacissima* leaves may cause cytotoxicity and genotoxicity. These medicinal plant extracts must be used with extra caution because their effects may be toxic at the molecular and DNA level when used inappropriately.

Supplementary materials

Not applicable

Author contributions

Conceptualization, (S.Q.) and (A.A.); methodology, (S.Q.) and (A.A.); formal analysis, (S.Q.); (A.A.), (K.A.), (A.F.A.), (W.F.), (M.E.) and (A.Q.); investigation, S.H.Q. and A.B.A..; writing—original draft preparation, (S.Q.); (A.A.), (K.A.),(A.F.A.), (W.F.), (M.E.) and (A.Q.); writing—review and editing, (S.Q.); supervision, (S.Q.); project administration, (S.Q.); funding acquisition, (S.Q.) All authors have read and agreed to the published version of the manuscript

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Data Availability Statement

All data is available in the manuscript.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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