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# Exploring *Moringa oleifera* Leaf Extract as a Promising Radioprotective Agent: Comparative Evaluation with Amifostine in a *Pangasius sutchi* Model

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**Abstract:** Apart from treatment, radiotherapy left patients vulnerable to radiation damage. Such damage can be prevented through the synthetic drug amifostine, which may also cause adverse effects. In this regard, this study is focused on exploring the antioxidant activity of *Moringa oleifera* due to their high phenolic content, which neutralizes the free radicals. The phytochemical results showed TPC, TFC, and antioxidant activity were high in methanol extract followed by 30% ethanol extract, aqueous extract, and ethanol extract. HPLC results showed higher kaempferol content in all the extracts than in quercetin. Since methanol extract may cause toxic effects on living cells, the radioprotective ability was assessed using 30% ethanol extract of *M. oleifera* leaf (MOLE), which shows nearly similar activity to methanol extract. The cytotoxicity analysis was carried out using micronucleus assay and comet assay showing erythrocytic abnormalities and DNA damage respectively in erythrocytes of the piscine model "*Pangasius sutchi*" exposed to cobalt-60 ($Co_{60}$) gamma radiation. The results show that amifostine has a higher radioprotective effect, followed by MOLE and phenolic antioxidant compounds such as kaempferol and quercetin. Hence, the current study results in the efficiency of MOLE as a radioprotective agent as it shows less cytotoxicity similar to amifostine.

**Keywords:** amifostine, cytotoxicity, kaempferol, *Moringa oleifera*, quercetin

## Introduction

Radiotherapy is a successful cancer treatment, where the cells get sterilized or killed when exposed to ionizing radiation. Radiation causes damage to DNA within cancer cells, rendering them unable to divide and develop further. Although radiation is focused on the tumor, it is unavoidable that it will also harm and destroy the normal, noncancerous surrounding tissue (Cinkilic et al., 2014). Radiolytic disintegration of cellular water creates free radicals, which can potentially cause lesions in cells and tissues through direct and indirect actions, causing damage to various biocomponents such as lipids, proteins, single-strand and double-strand damage in DNA (Kamran et al., 2016).

Hence, research has been conducted to reduce damage in normal tissue utilizing synthetic substances such as amifostine. However, it is hazardous when provided at the high dosages necessary for radioprotection (survival benefits). As a result, this promising agent has been deemed unsuitable for use as a radioprotector in special operations troops, high-risk personnel, or the general civilian population (Singh and Seed, 2019). Due to the limits of present radioprotective chemicals, a significant effort has been undertaken to find less harmful compounds. The potential use of natural phenolic compounds as radioprotectants is gaining attention because of their known antioxidant and free-radical scavenging properties (Cinkilic et al., 2014). Plant extracts are utilized as a substitute for synthetic antioxidants in treating various ailments because of their therapeutic value, affordability, and minimal toxicity (Gómez-Martínez et al., 2020).

*M. oleifera* has a broad spectrum of medicinal and therapeutic effects in lowering oxidative stress (Kou et al., 2018) and heavy metal chelating activities due to its richness of antioxidant phytochemicals (Valdez-Solana et al., 2015). The primary flavonoids found in the leaf of *M. oleifera* were kaempferol and quercetin (Rodríguez-Pérez et al., 2015), which exhibit identical antioxidant properties when compared. Kaempferol has several pharmacological characteristics and is used in cancer treatment. Indeed, kaempferol protects non-mutant cells while inducing apoptosis in mutated cells (Imran et al., 2019). Quercetin has strong antioxidant effects since it can effectively neutralize free radicals and bind metal ions via chelation (Yang et al., 2020). Therefore, this work aims to discover a naturally occurring radioprotective compound by comparing it with synthesized medications using cytogenetic assays in the erythrocytes of *P. sutchi*.

## Material and methods

### Collection and identification of plant material

The *Moringa oleifera* leaf was collected from Thaiman organic farms, Kannigapuram, Anaicut, Vellore District (12°52'50.5"N, 79°00'12.3"E), Tamil Nadu, India.

### Preparation of the leaf extracts

Methanol (SRL, India), 30% ethanol, distilled water, and ethanol (Changshu Hongshang Fine Chemicals, China) solvents were studied comparatively. The solvent-to-sample ratio of 10:1 (volume/weight) was used in this study (Pandey, Tripathi 2014). The extracts were prepared using Khan et al., (2017) protocol.

### Quantitative analysis of phytochemicals

### Flavonoid estimation

Flavonoid was estimated using an aluminum chloride (SRL, India) colorimetric test. The extract was replaced with methanol to make the blank and the standard curve of quercetin (Merck, Germany) was drawn at concentrations of 100-500 µg/mL (at the interval of 100 units). The absorbance of the sample and quercetin standards were calculated at 415 nm. The findings of the calibration curve are presented as quercetin equivalent in µg/mL of extract (García-Beltrán et al., 2020).

**Phenol estimation**

The Folin-Ciocalteau (SRL, India) reagent technique was used to measure the total phenol concentration. The absorbance at 725 nm was recorded and compared to the absorbance of the reagent blank (García-Beltrán et al., 2020). The reference standard was gallic acid (Merck, Germany), and its calibration curve was shown at various concentrations (100-500 µg/mL). The total phenolic quantity was calculated using the standard curve and represented as gallic acid equivalent in µg/mL of extracts (Roy et al., 2018).

**High-performance liquid chromatography (HPLC) analysis**

The known standard solution was made by mixing 0.5 mg of quercetin and kaempferol (Merck, Germany) in 1 mL of methanol. The solutions underwent filtration using a syringe filter with a pore size of 0.2 µm. Similarly, 5 mg of leaf extract was mixed in 1 mL methanol and filtered. It was then diluted to lower concentrations from this solution. The HPLC (Shimadzu LC-20AD, Japan) analyzer was used to identify the amount of phytocompounds present in the plant sample. The phenolic compounds were separated using the $C_{18}$ column. A mobile phase consisting of a mixture of acetonitrile (50%) (HI media, India) and methanol (50%) was used at a flow rate of 1.0 mL/minute at room temperature. The wavelength utilized for the analysis was 254 nm and the peak area of the compound was compared with the standard component (Sánchez-Machado et al., 2010). The elution was carried out using a gradient solvent system consisting of 0.1% formic acid (SRL, India) (solvent A) and acetonitrile (solvent B) as mobile phases with the following ratios: 80:20 (A/B) for 3 minutes, 80:20 to 65:35 (A/B) in 11 minutes, held for 14 minutes, 65:35 to 0:100 (A/B) in 25 minutes, and held for 5 minutes. For all the samples and standards, the injection volume was 20 µl (Vongsak et al., 2014).

**Antioxidant assay**

The antioxidant activity of the plant extract at various concentrations (100, 200, 300, 400, and 500 µg/mL) was assessed for their 2,2-diphenylpicrylhydrazyl (DPPH) (HI media, India) free radical scavenging activity (Luciana L. Mensor et al., 2001), and ferric-reducing antioxidant power (FRAP) (Saleem et al., 2020). The positive control-ascorbic acid (HiMedia, India) was parallelly assessed for its scavenging activity to compare with plant extract

**Acclimatization of fish**

A single breed of *P. sutchi* fingerlings measuring around 7.00 ± 0.5 cm in length and weighing approximately 10.00 ± 1.00 g were obtained from ASM aquarium, Chennai, Tamil Nadu, India. The physical and chemical parameters of the tank water and fish were maintained according to OECD (2019) guidelines. The fixed-dose technique recommended by the "British Toxicology Society" was followed to reduce animal numbers in this research (Stallard & Whitehead, 1995).

**Ethics approval**

The procedures were performed according to CPCSEA (2021) guidelines for experimentation on fish. Approval was granted by the institutional ethical committee for animals, Center for Environmental Nuclear Research, SRM Institute of Science and Technology. Ethical registration number: 15/IAEC/CENR/04

**Identification of $LD_{50}$ of γ-irradiation in Fish:**

The fish were segregated into eight groups each containing 8 fish (one group is control and seven are irradiated). The irradiation was performed at a dose ranging from 5.0 to 20 Gy at the interval of 2.5 Gy units. The $LD_{50}$ of γ-irradiation in *P. sutchi* was determined by subjecting the fish using a $Co^{60}$ gamma irradiator apparatus (gamma chamber-5000, India). The irradiated fish were maintained for 32 days and the number of dead fish was recorded to find the radiation dose responsible for 50% mortality.

**Identification of low observed effective level (LOEL) for 96 hours**

The LOEL level of amifostine (Amifosted-500, Therdose pharma, India), 30% ethanol extract of *M. oleifera* leaf (MOLE), kaempferol, and quercetin was measured in the erythrocytes of *P. sutchi* by comet assay. The intra-muscular injection of each compound was given in six different doses (8 fish/dose group) to find the LOEL concentration. The fish were sedated using clove oil (Himedia, India) (20 mg/L) after being randomly selected from the test tanks (CPCSEA, 2021). A total of 0.1mL of blood was extracted from the caudal vein at a rate of 2 fish per time (Acar et al., 2018). The collected blood was carefully transferred into potassium EDTA additive vacuum tubes (Becton Dickinson India Private Limited). Based on the percentage of DNA tail, the LOEL concentrations of these components were determined for radioprotective activities.

**Radio-protection studies**

A radioprotective study was conducted in *P. sutchi* using LOEL concentration of amifostine, MOLE, kaempferol, and quercetin with an $LD_{50}$ dose of γ-irradiation. The fish were divided into six groups, with ten fish in each group as given below, (i) PBS control, (ii) $LD_{50}$ gamma irradiation, (iii) $LD_{50}$ gamma irradiation +

amifostine, (iv) $LD_{50}$ gamma irradiation + MOLE, (v) $LD_{50}$ gamma irradiation + kaempferol, (vi) $LD_{50}$ gamma irradiation + quercetin

**Micronucleus (MN) Assay**

The MN assay was carried out using Bolognesi and Hayashi (2011) methodology. A thin smear of blood samples was done on a clean glass slide (Borosil, India), allowed to dry in the air, and then treated with methanol for 15 minutes. Each slide was stained with a 5% Giemsa solution (SRL, India) for 20 minutes. At least 1,000 erythrocytes were identified from each slide using 40× magnification of the inverted bright field microscope (Leica, Germany). The nuclear anomalies were categorized under four classifications (Sinha et al., 2018a), such as (i) micronuclei – small nucleus, (ii) binucleated nucleus - two distinct nuclei in the cytoplasm of a single erythrocyte, (iii) vacuolated cells – small fluid-filled spaces within the cytoplasm, and (iv) apoptotic cells – cells undergoing apoptosis.

**Comet Assay**

10 µL of blood is mixed with 90 µL of low melting point agarose (Himedia, India), fixed in a high agarose-coated (Himedia, India) slide, and kept in lysis solution to denature the membrane and unwind the damaged DNA. After that, the samples are electrophoresed at 22-24 V, and stained with a fluorescent dye (ethidium bromide – SRL, India). The slide was observed under 20× of fluorescent microscopy (Carl Zeiss, AXIO, Germany) to examine the damaged DNA (Singh et al., 1988). The bandpass filter for excitation is 546/10 nm, and for emission is 590 nm. The damaged DNA images were processed using Open Comet Software (v1.3.1) to determine the proportion of DNA tail damage.

**Statistical Analysis**

The graphing and statistical analysis was carried out using Origin Pro software (version 2024). One-way ANOVA was carried out for the significance of group means at 0.05 level. The Fisher test made the mean comparison, and the homogeneity of variance test was done by Levene's test (Absolute Deviations).

**Results and Discussion**

**Quantitative analysis**

The quantitative analysis of plant extract estimated total phenolic content (TPC) and total flavonoid content (TFC). The methanol extract showed higher phenolic content with 0.477 µg/mL, followed by 30% ethanol extract (0.364 µg/mL), aqueous extract (0.230 µg/mL), and ethanol extract (0.155 µg/mL). The TFC analysis results with

high flavonoid content in methanol extract (0.209 µg/mL), followed by 30% ethanol extract (0.176 µg/mL), aqueous extract (0.120 µg/mL), and ethanol extract (0.087 µg/mL). The results are shown in Figure 1.

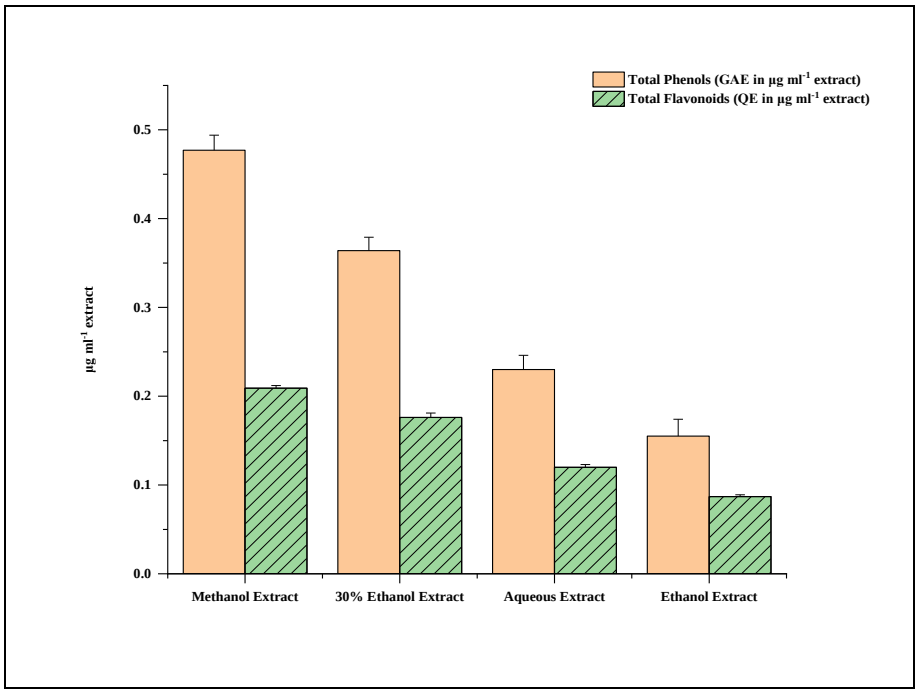


Figure 1. Quantitative analysis results show total phenolic and flavonoid content. The results are given as mean ± standard deviation.

**Antioxidant assay**

The DPPH antioxidant activity results are given in Figure 2. The sample results were compared with the standard ascorbic acid. Methanol extract showed higher scavenging activity, followed by 30% ethanol extract, aqueous extract, and ethanol extract. There was no broad difference between these polar solvents. The methanolic extract showed lower DPPH activity (62.07% at 100µg/mL concentration) compared to the report by Asgari-Kafrani et al., (2020) in *M. oleifera* leaf procured from Iran (75.45% at 80 µg/mL concentration). This shows that antioxidant activity may change from one region to another for the same plant species. Anjum Mobeen Syeda and K. Riazunnisa, (2020) showed similar DPPH results for standard ascorbic acid and methanolic extract of *M. oleifera* from Kadapa, India. Among the solvents used in this study, methanol extract showed better scavenging activity than other solvents.

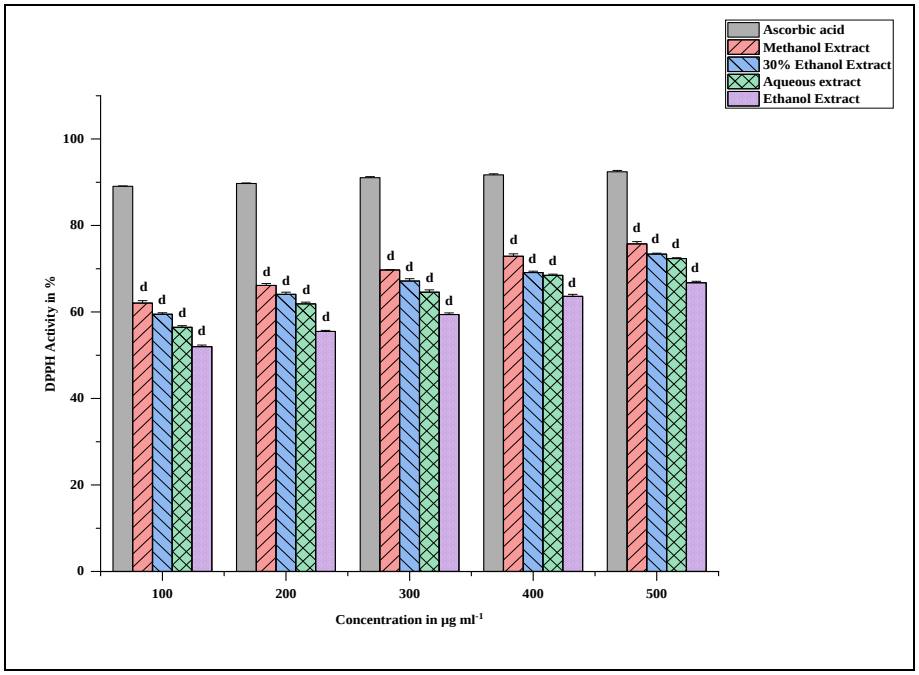


Figure 2. The percentage of DPPH scavenging activity of various polar solvents is shown in the graph. The results are given as mean ± standard deviation. The one-way ANOVA (Fisher's test) result for the DPPH assay shows that the group means showing the letter "d" is significantly different at $p < 0.0001$ level compared to standard ascorbic acid.

The FRAP results are given in Figure 3. Similar to the DPPH assay, ascorbic acid was taken as standard. However, the values differ due to different assay protocols. Methanol extract shows higher activity, followed by 30% ethanol, aqueous and ethanol extract. This study shows a significant difference in activity between these polar solvents.

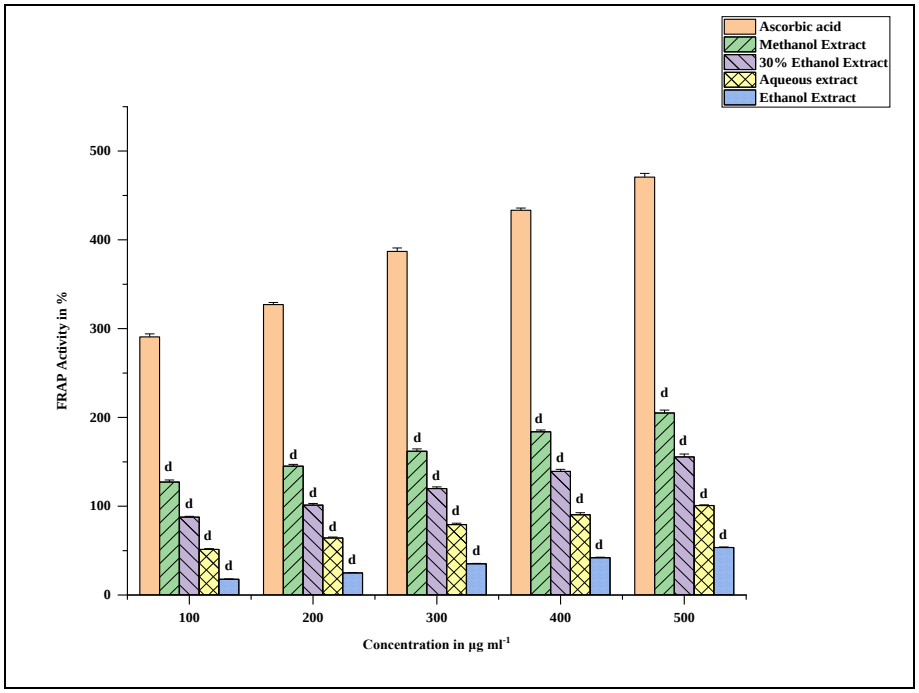


Figure 3. The Percentage of FRAP scavenging activity of various polar solvents is given as mean ± SD. The one-way ANOVA (Fisher's test) result for FRAP assay shows that the group means showing the letter "d" is significantly different at $p < 0.0001$ level compared to standard ascorbic acid.

**HPLC analysis**

The samples were analyzed using quercetin and kaempferol as standards, due to the high availability of these phenolic compounds in any plant extract. The quantity of kaempferol was high in methanol extract (0.044 µg/mL) followed by 30% ethanol extract (0.028 µg/mL), aqueous extract (0.021 µg/mL), and ethanol extract (0.0029 µg/mL). The amount of quercetin was high in methanol extract (0.0236 µg/mL) followed by 30% ethanol (0.0125 µg/mL), aqueous extract (0.0047 µg/mL), and ethanol extract (0.0019 µg/mL). The differences in kaempferol and quercetin content between extracts of the same plant leaf may be due to the polarity of solvents, which is responsible for the extraction of antioxidant phenolic compounds. Figure 4 shows the spectrum of standards and samples.

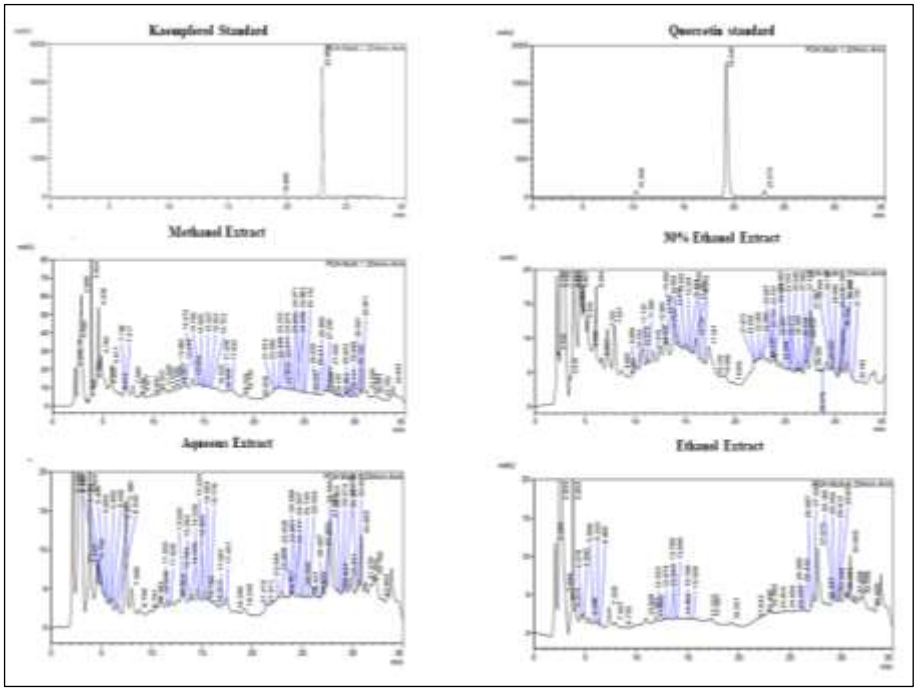


Figure 4. HPLC graphs showing the standard peak of kaempferol, quercetin, and the peaks in different polar solvent extract separately.

**Determination of $LD_{50}$ of γ-irradiation in *P. sutchi***

The $LD_{50}$ of γ-irradiation was 15 Gy, where 50% of fish died at this dose. Finney's probit statistical analysis shows the accurate $LD_{50}$ of irradiation in the *P. sutchi* fish population, and it was 14.45 Gy, which was higher than the results reported by Sinha et al., (2018b), who found 10.2 Gy as $LD_{50}$ for the same fish variety. The difference in $LD_{50}$ dose among the same animal species may be due to various factors such as age, size, acclimatization condition of fish, or the reduction of γ-emission concerning the half-life of the source $Co^{60}$, which in turn increases the exposure period to attain lethal dose since the gamma chamber emits radiation based on calculating the emission rate of the instrument at that time.

**Determination of LOEL by comet assay**

The comets are categorized based on size, fluorescence intensity, and tail length (i) no damage/or minor damage (ii) minimal damage (iii) tail length between two to three-fold the diameter of the nucleus and (iv) significant damage with a tail length greater than three-fold the diameter of the nucleus (Zhang et al., 2018). The representative image of the comet-DNA tail is shown in Figure 5 A. The analyzed image of the comet using "Open Comet" software is given in Figure 5 B. The LOEL concentration was found to be 80 mg/Kg body weight (B.W.) for amifostine (Figure 6), 130 mg/Kg B.W. for MOLE (Figure 7), and 20 mg/Kg B.W. for both kaempferol and quercetin (Figure 8). These results were estimated based on the DNA tail damage of around 10% for comet

assay. Below this percentage was considered a null effect or the natural apoptotic phase of cells. The DNA tail damage higher than 10% reveals the negative effect of a higher concentration of the drug.

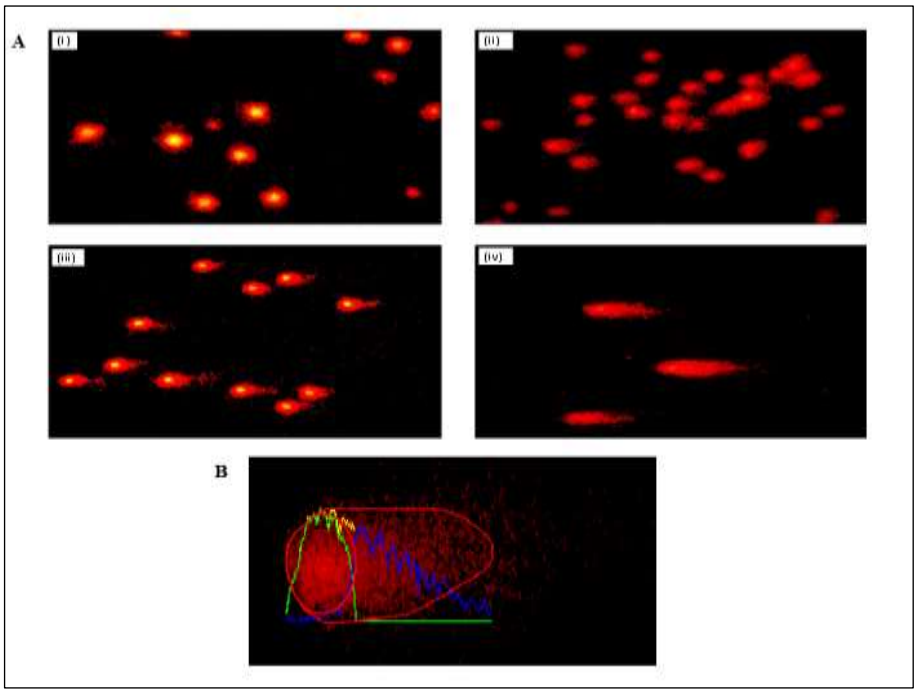


Figure 5. A - Representative image of comets based on size and tail length (i) no damage/or minor damage (ii) minimal damage (iii) tail length between two to three-fold the diameter of the nucleus and (iv) significant damage with a tail length greater than three-fold the diameter of the nucleus. B - The representative analyzed image of the comet cell using "open comet" software showing head and tail regions.

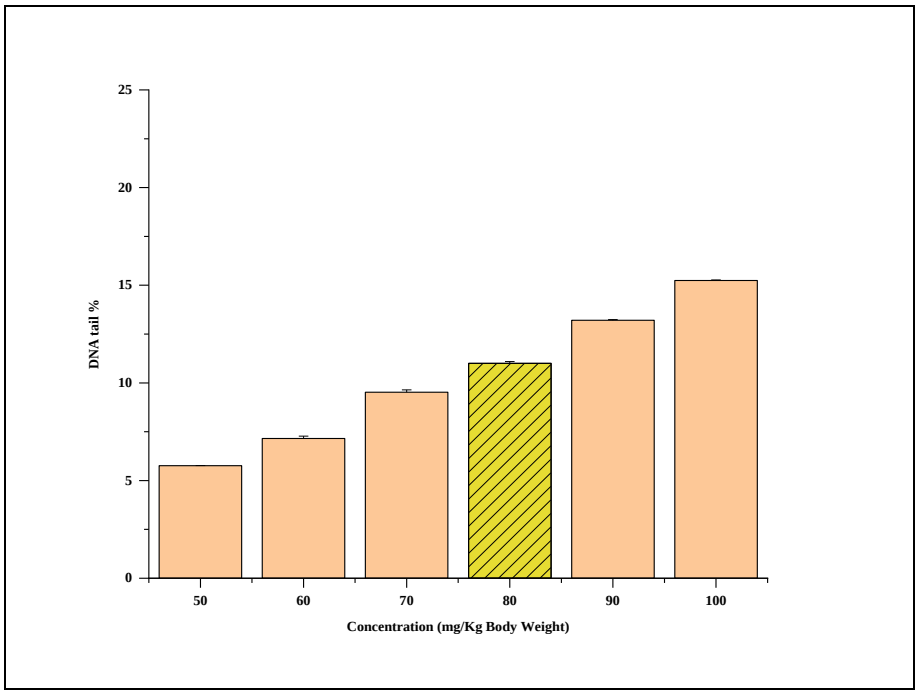

Figure 6. LOEL concentration for amifostine based on the percentage of DNA tail. The results are given as mean ± standard deviation.

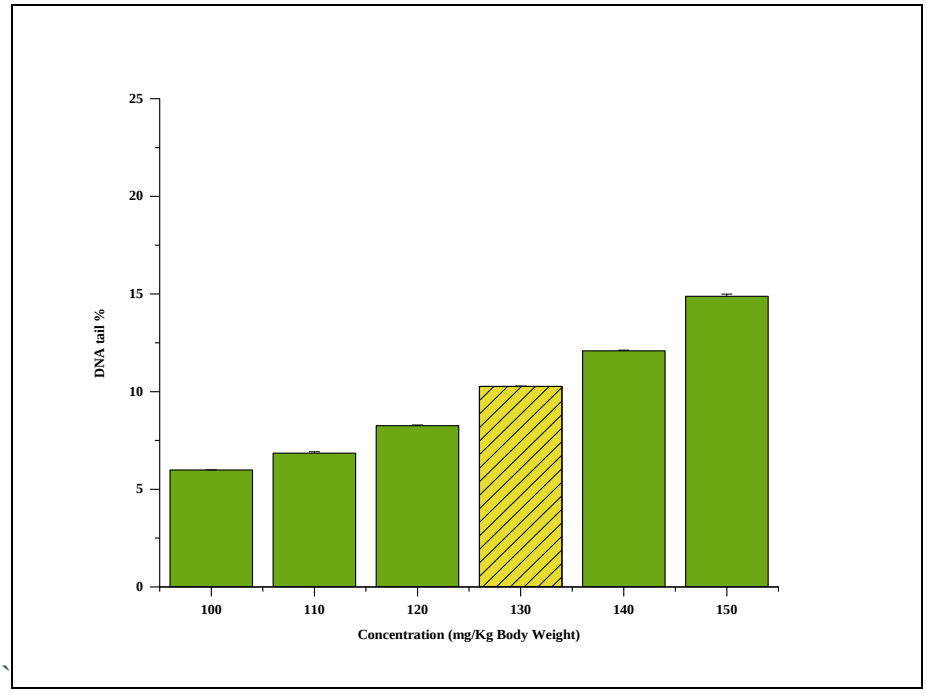


Figure 7. LOEL concentration for MOLE based on the percentage of DNA tail. The results are given as mean ± standard deviation.

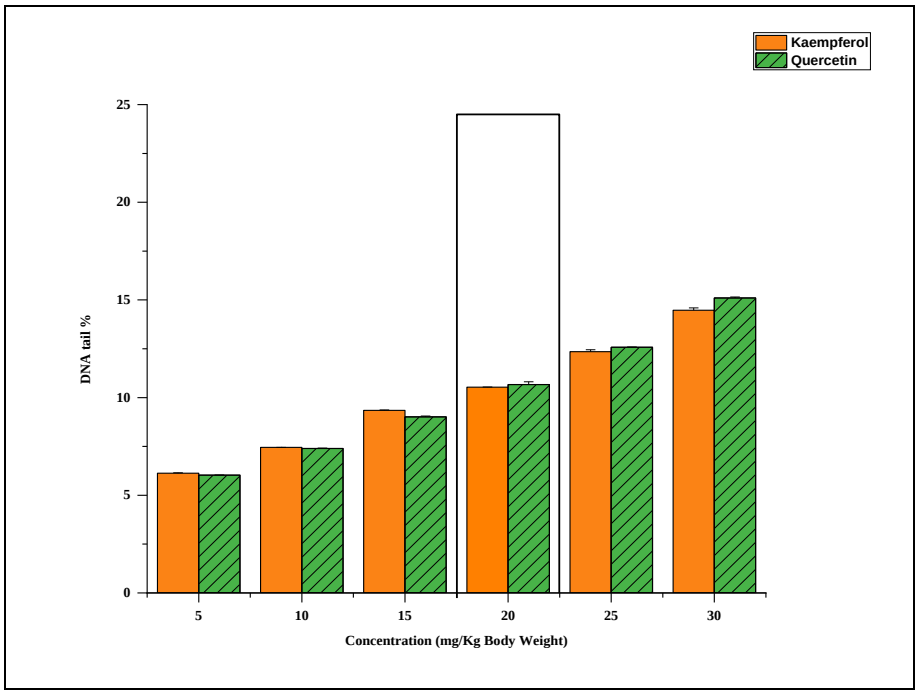

Figure 8. LOEL concentration for kaempferol, and quercetin based on the percentage of DNA tail. The results are given as mean ± standard deviation.

**Radio-protection studies**

The results for radioprotection studies analyzed using comet and MN assay were given in Figures 9 and 10 by calculating its DNA tail percentage and erythrocytic abnormality percentage, respectively in *P. sutchi* pre-administered with LOEL concentration of compounds and exposed to $LD_{50}$ of γ-irradiation. The representative image for erythrocytic abnormalities is shown in Figure 11.

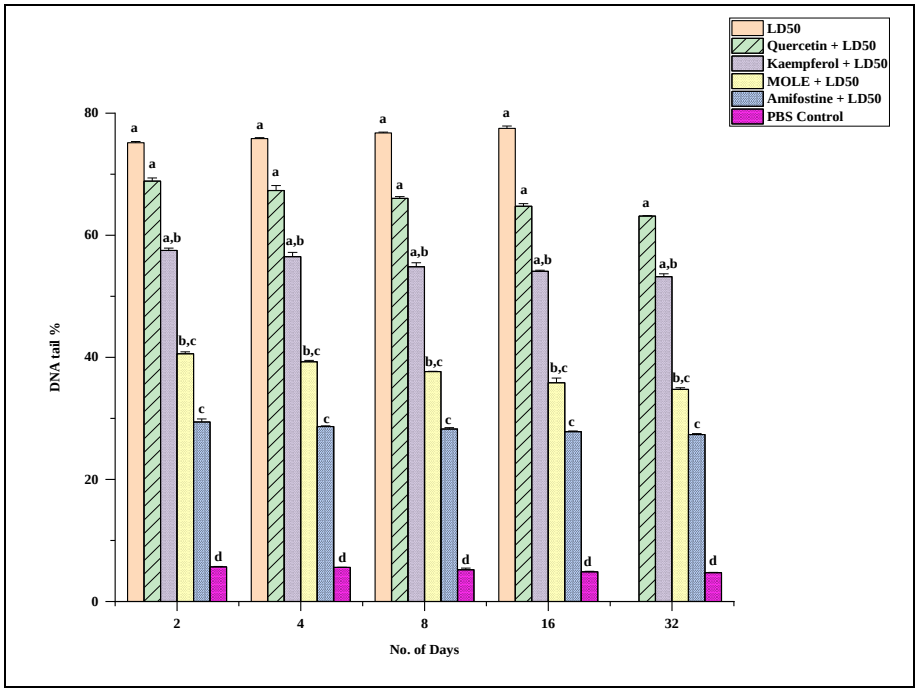


Figure 9. Radioprotection effects of amifostine, MOLE, kaempferol, and quercetin using comet assay in *P. sutchi* exposed to $LD_{50}$ of gamma irradiation. The one-way ANOVA (Fisher's test) results show that the group means that do not share a similar letter are significantly different at 0.05 level. The homogeneity of variances (Levene's test – Absolute deviations) shows the group variances are significantly different at 0.05 level

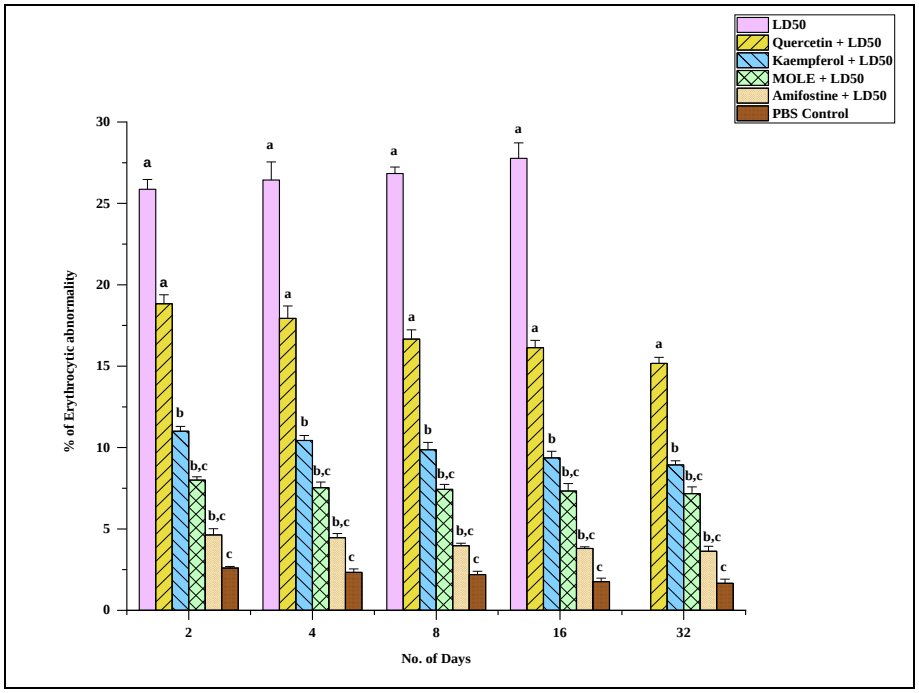


Figure 10. Radioprotection effect of amifostine, MOLE, kaempferol and quercetin using micronucleus assay in *P. sutchi* exposed to $LD_{50}$ of gamma irradiation. The one-way ANOVA (Fisher's test) results show that the group means that do not share a similar letter are significantly different at $p \leq 0.05$ level. The Homogeneity of variances (Levene's test – Absolute deviations) shows the group variances are significantly different at $p \leq 0.05$ level

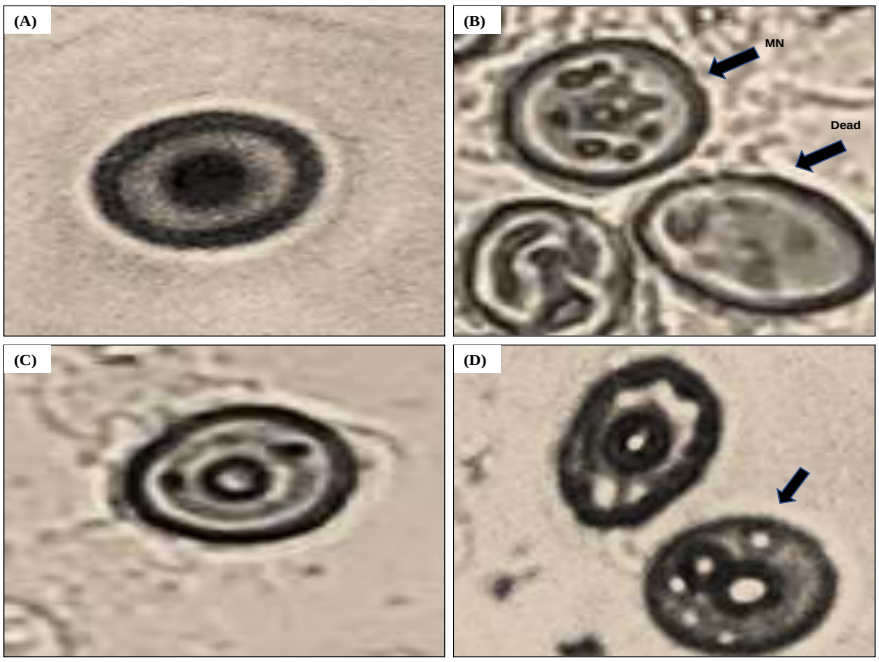


Figure 11. The erythrocytic abnormalities were categorized under four groups, such as: (a) - normal cell, (b) – micronucleated and apoptotic cell, (c) - binucleated cell, and (d) - vacuolated cell.

In this experiment, the higher DNA tail percentage and erythrocytic abnormality percentage are inversely proportional to healthy cells or the radioprotective effect. The $LD_{50}$ group shows an increase in DNA tail percentage from 75.15% ($2^{nd}$ day) to 77.51% ($16^{th}$ day) and erythrocytic abnormality percentage from 25.86% ($2^{nd}$ day) to 27.76 % ($16^{th}$ day) due to the multiplication of free radicals damaging the tissues and lack of antioxidant mechanism taking place in animal body post-irradiation. The experiment was terminated for this group on the $16^{th}$ day since no animals were left. The other groups treated with amifostine, MOLE, kaempferol, and quercetin showed a decrease in DNA tail percentage and erythrocytic abnormality percentage as the days passed. The lower DNA tail percentage was seen in the amifostine group (29.41% on $2^{nd}$ day and 27.36% on $32^{nd}$ day), followed by MOLE (40.57% on $2^{nd}$ day and 34.76% on $32^{nd}$ day), kaempferol (57.51% on $2^{nd}$ day and 53.22% on $32^{nd}$ day), and quercetin groups (68.88% on $2^{nd}$ day and 63.15% on $32^{nd}$ day). Similar to the comet assay, the MN assay also shows a decrease in erythrocytic abnormality percentage for amifostine (4.63% on $2^{nd}$ day and 3.63% on the $32^{nd}$ day), followed by MOLE (8.00% on $2^{nd}$ day and 7.16% on $32^{nd}$ day), kaempferol (11.00% on $2^{nd}$ day and 8.93% on $32^{nd}$ day) and quercetin groups (18.83% on $2^{nd}$ day and 15.16% on $32^{nd}$ day), since these antioxidant compounds give radioprotective effects pre-irradiation and enhance the cell rejuvenation and DNA repair mechanism post-irradiation when compared with PBS control (5.67% on the $2^{nd}$ day and 4.73% on the $32^{nd}$ day in comet assay and 2.60% on the $2^{nd}$ day and 1.66% on the $32^{nd}$ day in MN assay). As with the same, the PBS control group shows a slightly increased DNA tail percentage and erythrocytic abnormality percentage in the early days and becomes normal as days go on because of the minimal stress to animals due to injection of PBS as well during travel to radiation facility.

The comet and micronucleus tests conducted using fish erythrocytes may be used to discover extrachromosomal DNA adducts in vivo caused by strand breaks, chromosomal damage, or aneugenic effects. These tests often exhibit a strong correlation; however, variations may arise owing to discrepancies in genotoxic mechanisms or DNA repair capability. For instance, the comet test may detect DNA damage, which can then be repaired by intracellular repair mechanisms. However, the micronucleus test only detects DNA lesions not repaired by the cell's machinery (Suares Rocha et al., 2022). The appearance of micronuclei, binucleate, vacuolated, and dead cells indicate structural or numerical chromosomal abnormalities during mitosis and cytokinesis blockage, which causes a genetic disproportion in the cells and leads to carcinogenesis (Sinha et al., 2018a).

The findings of Wang et al., (2018) showed that kaempferol (15 mg/Kg B.W.) can successfully increase the 30-day survival rate in mice following 8.5 Gy (TBI) by lowering oxidative stress and minimizing morphological changes and cell death. These results closely relate to our current 20 mg/Kg B.W. study in *P. sutchi* following 14.45 Gy of ionizing radiation (IR). Hodhod et al., (2019) discovered that post-treatment with *M. oleifera* leaf

extract lowers the lethal effects of radiation exposure without affecting the experimental rats' hematological, behavior, or body weight.

The results showed that the medically available and currently using sulfhydryl compound amifostine has a higher radioprotective effect, followed by MOLE, kaempferol, and quercetin. However, the plant extract shows a radioprotective nearing impact with the synthetic drug amifostine, which may be due to a mixture of phytochemicals arising from synergistic processes, enhancing cascade mechanisms in *M. oleifera* leaf when compared with singlet antioxidant flavonoids such as kaempferol and quercetin. Hence the present study leads to the development of herbal drugs in radiotherapy.

## Conclusion

Until now, only one US FDA-approved radioprotective drug is available for human use, which necessitates substantial research in finding the phytocompounds towards using effective radioprotective compounds without any side effects. The current study results in the efficiency of MOLE as a radioprotective agent as it shows less DNA damage and erythrocyte abnormalities in a 32-day study similar to the synthetic drug amifostine. The cascade mechanism of plant extract, which gives more protection than single antioxidant phenols, must be studied further using other phytochemicals in *M. oleifera.* It was also demonstrated that micronucleus and comet assays might be used rapidly, making them viable test biomarkers to detect radiation-induced damage in fish.

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## References

1. Acar, Ü., İnanan, B.E., Zemheri, F., Kesbiç, O.S. and Yılmaz, S. (2018). Acute exposure to boron in Nile tilapia (*Oreochromis niloticus*): Median-lethal concentration (LC50), blood parameters, DNA fragmentation of blood and sperm cells. *Chemosphere.* 213: 345–350. https://doi.org/10.1016/j.chemosphere.2018.09.063
2. Asgari-Kafrani, A., Fazilati, M. and Nazem, H. (2020). Hepatoprotective and antioxidant activity of aerial parts of *Moringa oleifera* in prevention of non-alcoholic fatty liver disease in Wistar rats. *South African Journal of Botany*. 129: 82-90. https://doi.org/10.1016/j.sajb.2019.01.014

3. Bolognesi, C. and Hayashi, M. (2011). Micronucleus assay in aquatic animals. *Mutagenesis.* 26(1): 205-213. https://doi.org/10.1093/mutage/geq073
4. Cinkilic, N., Tüzün, E., Çetintaş, S.K., Vatan, Ö., Yılmaz, D. et al. (2014). Radio-protective effect of cinnamic acid, a phenolic phytochemical, on genomic instability induced by X-rays in human blood lymphocytes in vitro. Mutation research. *Genetic toxicology and environmental mutagenesis.* 770: 72-79. https://doi.org/10.1016/j.mrgentox.2014.04.025
5. CPCSEA (2021). Guidelines of CPCSEA for Experimentation on Fish. Government of India Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying. https://ccsea.gov.in/
6. García-Beltrán, J.M., Mansour, A.T., Alsaqufi, A.S., Ali, H.M. and Esteban, M.Á. (2020). Effects of aqueous and ethanolic leaf extracts from drumstick tree (*Moringa oleifera*) on gilthead seabream (*Sparus aurata* L.) leucocytes, and their cytotoxic, antitumor, bactericidal and antioxidant activities. *Fish Shellfish Immunol.* 106: 44-55. https://doi.org/10.1016/j.fsi.2020.06.054
7. Gómez-Martínez, M. et al. (2020). Location and tissue effects on phytochemical composition and in vitro antioxidant activity of *Moringa oleifera*. *Ind Crops Prod.* 151:112439. https://doi.org/10.1016/j.indcrop.2020.112439
8. Hodhod, T.E., Ammar, N.M., Hassan, H.A., Orban, H.A. and El-Baz, H.A. et al. (2019). The radioprotective effects of methanolic extract of *Moringa oleifera* leaves against gamma irradiation in rats. *Bioscience Research.* 16(1):428-437 Hodhod et al., (isisn.org).
9. Imran, M., Salehi, B., Sharifi-Rad, J., Aslam Gondal, T., Saeed, F. et al. (2019). Kaempferol: A Key Emphasis to Its Anticancer Potential. *Molecules.* 24(12), 2277. https://doi.org/10.3390/molecules24122277
10. Kamran, M.Z., Ranjan, A., Kaur, N., Sur, S. and Tandon., V. (2016). Radioprotective Agents: Strategies and Translational Advances. *Medicinal Research Reviews,* 36(3), 461–493. https://doi.org/10.1002/med.21386
11. Khan, W., Parveen, R., Chester, K., Parveen, S. and Ahmad, S. (2017). Hypoglycemic Potential of Aqueous Extract of *Moringa oleifera* Leaf and In Vivo GC-MS Metabolomics. *Front. Pharmacol.* 8:577. https://doi.org/10.3389/fphar.2017.00577
12. Kou, X., Li, B., Olayanju, J.B., Drake, J.M. and Chen, N. (2018). Nutraceutical or Pharmacological Potential of *Moringa oleifera* Lam. *Nutrients.* 10(3): 343. https://doi.org/10.3390/nu10030343
13. Luciana, L. Mensor. et al. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research.* 15: 127-130. https://doi.org/10.1002/ptr.687
14. OECD (2019). Test No. 203: Fish, Acute Toxicity Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. https://doi.org/10.1787/9789264069961-en

15. Pandey, A. and Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug, *Journal of Pharmacognosy and Phytochemistry.* 2(5), 115-119. https://www.phytojournal.com/
16. Rodríguez-Pérez, C., Quirantes-Piné, R., Fernández-Gutiérrez, A. and Segura-Carretero, A. (2015). Optimization of extraction method to obtain a phenolic compounds-rich extract from *Moringa oleifera* Lam leaves. *Ind Crops Prod.* 66: 246-254. https://doi.org/10.1016/j.indcrop.2015.01.002
17. Roy, A.R. (2018). Qualitative and Quantitative Phytochemical Analysis of *Centella asiatica*. *Nat Prod Chem Res.* 06, Issue 4, 323. https://doi.org/10.4172/2329-6836.1000323
18. Saleem, A., Saleem, M. and Akhtar, M.F. (2020). Antioxidant, anti-inflammatory and antiarthritic potential of *Moringa oleifera* Lam: An ethnomedicinal plant of Moringaceae family. *South African Journal of Botany.* 128: 246-256. https://doi.org/10.1016/j.sajb.2019.11.023
19. Sánchez-Machado, D.I, Núñez-Gastélum, J.A., Reyes-Moreno, C., Ramírez-Wong, B. and López-Cervantes, J. (2010). Nutritional quality of edible parts of *Moringa oleifera*. *Food Anal Methods*. 3: 175-180. https://doi.org/10.1007/s12161-009-9106-z
20. Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental cell research*. 175(1): 184-191. https://doi.org/10.1016/0014-4827(88)90265-0
21. Singh, V.K. and Seed, T.M. (2019). The efficacy and safety of amifostine for the acute radiation syndrome. *Expert opinion on drug safety.* 18(11): 1077-1090. https://doi.org/10.1080/14740338.2019.1666104
22. Sinha, P., Arunachalam, K.D. and Annamalai, S.K. (2018a). Radio-protective dosimetry of *Pangasius sutchi* as a biomarker, against gamma radiation dosages perceived by genotoxic assays. *Ecotoxicology and environmental safety.* 164: 629-640. https://doi.org/10.1016/j.ecoenv.2018.08.071
23. Sinha, P., Arunachalam, K.D. and Annamalai, S.K. (2018b). A Wee Study on Behavioural, Organ Somatic Index and Histological Alterations of the Fresh Water Fish *Pangasius sutchi* in Response to Protection Studies, Exposed to Gamma Radiation Perceived by Genotoxic Assays. *Int. J. Pharm. Sci. Rev. Res.* vol. 50. https://www.researchgate.net/publication/325845976.
24. Stallard, N. and Whitehead, A. (1995). Reducing animal numbers in the fixed-dose procedure. *Human & Experimental Toxicology.* 14 (4): 315-323. https://doi.org/10.1177/096032719501400401
25. Suares Rocha, P., Deutschmann, B. and Hollert, H. (2020). In Situ Determination of Genotoxic Effects in Fish Erythrocytes Using Comet and Micronucleus Assays. *Methods in Pharmacology and Toxicology.* 115-129. Humana, New York, NY. https://doi.org/10.1007/7653_2019_40

26. Syeda, A.M. and Riazunnisa, K. (2020). Data on GC-MS analysis, in vitro anti-oxidant and anti-microbial activity of the *Catharanthus roseus* and *Moringa oleifera* leaf extracts. *Data in brief.* 29: 105258. https://doi.org/10.1016/j.dib.2020.105258
27. Valdez-Solana, M.A. et al. (2015). Nutritional content and elemental and phytochemical analyses of *Moringa oleifera* grown in Mexico. *J Chem.* Article ID 860381, 9 pages. https://doi.org/10.1155/2015/860381
28. Vongsak, B., Sithisarn, P. and Gritsanapan, W. (2014). Simultaneous HPLC quantitative analysis of active compounds in leaves of *Moringa oleifera* lam. *J Chromatogr Sci.* 52: 641-645. https://doi.org/10.1093/chromsci/bmt093
29. Wang, J., Li, T., Feng, J., Li, L., Wang, R. et al. (2018). Kaempferol protects against gamma radiation-induced mortality and damage via inhibiting oxidative stress and modulating apoptotic molecules in vivo and vitro. *Environmental toxicology and pharmacology*. 60: 128-137. https://doi.org/10.1016/j.etap.2018.04.014
30. Yang, D., Wang, T., Long, M. and Li, P. (2020). Quercetin: Its Main Pharmacological Activity and Potential Application in Clinical Medicine. *Oxidative medicine and cellular longevity*. 8825387. https://doi.org/10.1155/2020/8825387
31. Zhang, M., Cao, G., Guo, X., Gao, Y., Li, W. and Lu, D. (2018). A comet assay for DNA damage and repair after exposure to carbon-ion beams or X-rays in Saccharomyces cerevisiae. *Dose-response*, 16 (3), 1559325818792467. https://doi.org/10.1177/1559325818792467