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IN VITRO NEPHROPROTECTIVE POTENTIAL OF ETHANOLIC LEAF EXTRACT OF *MIMUSOPS ELENGI*

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Abstract

Mimusops elengi plants have been utilized in traditional medicine from ancient time for the ailment of various diseases. The current study focuses on the phytochemical analysis of the ethanolic leaf extract and its antibacterial, antioxidant, and *in vitro* nephroprotective activity. These studies were conducted using *in vitro* methods and are given and discussed in this paper. The ethanolic leaf extract was subjected to phytochemical examination, which identified the presence of noteworthy phytoconstituents. The antioxidant activity of the extract was assessed through *in vitro* assays, specifically the (1,1-diphenyl picrylhydrazyl) radical scavenging assay. And for the antimicrobial assessment, respectively. The ethanolic leaf extract of *Mimusops elengi* has demonstrated strong protective activity against free radicals, as evidenced by results collected from antioxidant and proteinase inhibition tests. These findings highlight its crucial function in preserving the integrity of the cell membrane. The encouraging outcomes achieved from the *in vitro* assessment of antioxidant properties led us to investigate the potential of these plants in protecting the kidneys utilizing epifluorescence and cytoprotective assays. HEK 293 cell line, consisting of normal kidney cells, was employed for epifluorescence dual labelling and cytoprotective assay. The results demonstrated a noteworthy level of activity in comparison to the conventional antibiotic gentamycin. The data indicates that the chosen *Mimusops elengi* ethanolic leaf extract has the potential to be used as a beneficial antioxidant, antibacterial, anti-inflammatory, and nephroprotective agent for humans.

Keywords: *Mimusops elengi*, HEK 293 Cell line, antimicrobial, antioxidant.

INTRODUCTION

Nephrotoxicity, the prevailing renal issue, arises from the exposure of the body to a pharmaceutical agent or hazardous substance. Nephrotoxicity is characterized by reduced ability to concentrate urine, presence of tubular proteinuria, lysosomal enzymuria, and moderate glucosuria. It also leads to decreased ammonium excretion, reduced glomerular filtration rate, decreased creatinine clearance, elevated serum blood urea nitrogen, and morphological changes in kidney tissue. Various therapeutic drugs, chemicals, and heavy metals have the potential to harm the kidney, causing acute renal failure, chronic intestinal

nephritis, and nephritic syndrome, which can result in irreversible damage to the kidneys. Conversely, impaired kidney function also directly affects cardiovascular function. Gentamicin is an aminoglycoside antibiotic that is employed to treat serious infections. Traditionally, gentamicin's nephrotoxicity has been seen as a tubulopathy, where renal insufficiency is primarily caused by tubular destruction and malfunction. This may elucidate some clinical symptoms such as the presence of protein in the urine (proteinuria), the presence of enzymes in the urine (enzymuria), and changes in electrolyte levels (electrolytic alterations).(Al-Azzam *et al.* 2010; Tavafi 2013).

Herbal medicine, also referred to as ethnic medicine, is a traditional form of healthcare within the field of complementary and alternative medicine. It has been practiced worldwide for the prevention and treatment of physical and mental diseases. Currently, there is a growing global trend of utilizing natural goods, including herbal remedies, to cure both minor and severe illnesses. The exposure to dangerous substances, medication, or an imbalanced diet can lead to both acute and chronic nephrotoxicity. The renal proximal tubule has a rich blood supply, making it more prone to being affected by drug-induced kidney damage. Annually, almost 5 million individuals worldwide perish as a result of inadequate availability of essential treatments for renal diseases. Certain medications have the potential to cause kidney damage. Confirmed reports indicate that around 20% of renal symptoms have been caused by adverse medication reactions. According to Kim *et al.* (2012), the prevalence rate of nephrotoxicity is 66% among the elderly. Aminoglycoside antibiotics have been proven to cause nephrotoxic effects. Hence, their application is restricted to the treatment of severe Gram-negative infections. A recent clinical study by Khatoon *et al*. (2019) has confirmed the nephroprotective effects of many herbal medicines. Some examples of these medicines are Cryptocoryneauriculata and Annona reticulata. An in vivo investigation was conducted to examine the effects of the extracts from these medicinal herbs on the nephrotoxicity caused by gentamicin and cisplatin. The therapeutic impact was associated with the antioxidant efficacy of the extracts, as evaluated by the phenol concentration. (Tabatabaee 2011).

Oxygen-derived free radicals, including hydrogen peroxide, hydroxyl radicals, and superoxide anions, are highly harmful to cells and can lead to tissue damage. Excessive production of reactive oxygen species (ROS) can initiate bimolecular oxidation, leading to oxidative stress and ultimately causing cellular death. Furthermore, oxidative stress leads to inadvertent enzyme activation and damage to the oxidative system within cells. The reference is from Wiseman and Halliwell's work published in 1996. In the last thirty years, researchers have created treatments and formulas using antioxidants to cure and prevent complex diseases such as cancer and Alzheimer's disease. The present focus is on the natural presence of antioxidants and their significance in promoting human health and nutrition. The research on medicinal plants and vegetables provides convincing evidence that plant components possessing antioxidant activity can safeguard biological systems from oxidative stress. The medicinal capabilities of plants have been extensively explored in the context of recent scientific advancements worldwide, because to their potent pharmacological effects, low toxicity, and potential for commercial use.

M.elengi includes a diverse range of active phytoconstituents, which contribute to its different biological and pharmacological actions. The substance has a range of activities including antibacterial, antihemorroidal, antifungal, anticarcinogenic, free radical scavenging, antihyperglycemic, antineoplastic, gastroprotective, antinociceptive, and diuretic effects. It also exhibits antiviral and cytotoxic activities. The alcoholic extract of bakul has notable antiulcer activity in comparison to the petroleum ether extracts of bark. Satish *et al.* (2008) observed that they evaluated various extracts obtained from M. elengi leaves, including aqueous and different solvent extracts such as petroleum, ether, benzene, chloroform, methanol, and ethanol, for their antifungal properties using the poisoned food technique in vitro. The methanol and ethanol extracts dissolved in water showed a strong and statistically significant antifungal activity against all the fungi tested. The bark extract exhibited in vitro antimicrobial activity against Staphylococcus aureus, S. mutans, S. salivarius, S. sangius, Lactobacillus acidophilus, and Candida albicans. Additionally, the antibacterial activity of aqueous and solvent-based extracts from the aerial parts of Bakul was evaluated. (Prabhat *et al,* 2010,Deepak *et al,* 2005 and Katedeshmukh*et al,* 2010).

Hence, the primary objective of the present investigation is to assess the renal-protective efficacy of the leaf extract of *Mimusops elengi*, drawing upon established pharmacological research findings. This *in-vitro* study aims to align the rationale for the utilization of this herbal medication by individuals in India, based on its desired biological action.

Materials and Methods

Chemicals

Analytical standards of ethanol, DPPH, ascorbic acid, nutrient broth, Muller-Hinton agar, DMSO, ethidium bromide, and acridine orange. The biochemistry and microbiology labs of Sacred Heart College provided all the chemicals and reagents.

Collection and authentication of study plant

The *Mimusops elengi were* collected fromSacred Heart College campus, Tirupattur (District), Tamil Nadu (State), India. The plant was acknowledged and recognized as *Mimusops elengi* by Research officersDr. KN Sunil Kumar and Dr. P. Elankani, Siddha Central Research Insitute, Arumbakkam, Chennai, Tamil Nadu (Form No. PCOG002-ACF). The attained leaf sample was rinsed with tap water to eliminate dust and dried up until it was entirely dehydrated. The well-dried leaves were milled using an electric mixer and filtered through a typical flour filter.

Preparation of extract

The leaves are washed with distilled water and left to dry in the sun for a week. After a week, the shade-dried leaves are ground into fine powder and stored in an airtight container. For preparing the ethanolic extract, 5 grams of fine powder from the root were mixed with 50 ml of ethanol. Then the suspension is left to incubate for 48 hours, while occasional shaking is allowed. After 48 hours of incubation, the contents are filtered through Whatman No. 1 filter paper. Then the filtrate was collected separately and left for evaporation under sunlight. The thick ethanolic leaf residues were collected and stored in an airtight container for further

analysis. Whereas the freshly prepared raw leaf extract was used for analysis without any solvent.

Qualitative Phytochemical analysis

The plant extract was tested for detecting various phytochemicals present in the ethanolic extract of root and raw root sap of *Mimusopselengi*using the standard procedure prescribed by (Harborne, 1998); (kokate *et al.,* 2002).

1)Test for carbohydrates

a) Molish's test

Plant extract was treated with few drops of Alpha naphthol and concentrated sulphuric acid added to the mixture in a slanting position. Observance of violet colouringindicates the presence of carbohydrates.

b) Benedict's test

0.5 ml of extract was treated with 0.5 ml of Benedict's reagent and kept for water bath for 2 minutes. Formation of precipitates indicates the presence of carbohydrates

2)Test for tannins

a) Ferric chloride test

A small quantity of extract was mixed with water and heated in a water bath. The mixture was filtered and 5% of ferric chloride was added to the filtrate. A dark green colour was formed. It indicates the presence of tannins.

b) Lead acetate test

3 ml of 10% lead acetate was added to the sample solution. Formation of bulky white precipitate indicates the presence of tannins.

3)Test for saponins

2 ml of distilled water was added with the sample solution and shakes well. Formation of foams indicates the presence of saponins.

4) Test for alkaloids

a) Mayer's test

The sample solution is treated with 2 drops of Mayer's reagent. Formation of white creamy precipitate indicates the presence of alkaloids.

b) Wagner's test

Few drops of Wagner's reagent were added with the sample. Formation of reddish-brown precipitate indicates the presence of alkaloids.

5) Test for Flavonoids

The sample solution is treated with 1 ml of 2N sodium hydroxide. Formation of yellow colour indicates the presence of flavonoids.

6) Test for glycosides

The sample solution is treated with 3 ml of chloroform and 10% ammonia. Formation of pink colour indicates the presence of glycosides.

7) Test for quinones

1 ml of concentrated sulphuric acid is added to the sample in slanting position. Formation of red colour indicates the presence of quinones.

8) Test for Phenols

The sample solution is treated with few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols.

9) Test for terpenoids

The sample solution is added with 2 ml of chloroform and treated with concentrated sulphuricacid. Formation of red brown colour indicates the presence of terpenoids.

10) Test for steroids

Few drops of concentrated sulphuric acid are added to the sample solution in a slanting position, occurrence of brown ring indicates the presence of steroids.

Assay of Antioxidant activity by DPPH (1, 1-diphenyl-2- picrylhydrazyl) free radical scavenging activity

The free radical scavenging potential of the root sap and ethanolic root extract was determined using the method prescribed by Molyneux, (2004). The DPPH solution (0.006% w/v) was prepared in 95% ethanol. Different concentrations of the ethanolic root extract of *Mimusops elengi* (10, 20, 30, 40 and 50 µg/ml) was prepared in ethanol. 3 ml of different concentration of ethanolic leaf extract of *Mimusops elengi* extracts were mixed with 1 ml of DPPH solution in dark. Ascorbic acid, which is a strong antioxidizing agent is taken as standard, prepared in different concentrations using ethanol (10, 20, 30, 40 and 50 µg/ml). 3 ml of different concentration of standard solution of ascorbic acid was mixed with 1 ml of DPPH solution in dark.

The prepared solution of ascorbic acid and plant extracts samples were incubated for 30 minutes and then absorbance was measured using U.V. Spectrophotometer at 517 nm. Ethanol serves as a blank and the experiment was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula;

DPPH radical scavenging activity (%) = (Control OD-Sample OD) / Control OD x 100.

Antimicrobial activity

The antimicrobial test is an important technique used in pharmacology to study the efficacy and potency of antimicrobial agents from herbal extracts against microorganisms. The pathogenic microorganism chosen for the antimicrobial activities are *Bacillus subtilis, Enterococcus faecalis, Klebsiellapneumonia Pseudomonas aeruginosa*and *Candida albicans.*

Disc Diffusion method

Disc diffusion method was used for antimicrobial activity. A stock solution of extract was prepared by dissolving 0.1 g of extract with 100 mL of their respective solvents (distilled water and absolute ethanol) to produce a final concentration of 100 mg/mL. The stock solution was then diluted to concentrations of, 50,100 and 150 mg/mL of extract. 20 μL of each dilution was impregnated into sterile, blank discs 6 mm in diameter. 5 μL of extract was spotted alternately on both sides of the discs and allowed to dry before the next 5 μL was spotted to ensure precise impregnation. All discs were fully dried before the application on bacterial lawn. The positive controls used were tetracycline antibiotic discs all strains. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the discs. The assay was repeated trice. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the leaf extract.

Epifluorescence staining

A dual-staining procedure was used with epifluorescence microscopy, which allows the detection of live cells and dead cells. In this method, normal kidney cells (vero cells) were

suspended in 10 mL of phosphate-buffered saline (PBS), and 200 µL of the suspension was incubated with gentamicin, followed by the addition of 50 µL of the selected plant extracts at a concentration of 500 mg/mL. Another 200 µL of the suspension was incubated with gentamicin, followed by the addition of vitamin E, which is used as a positive control. To the above suspensions:50 µL of ethidium bromide and 50 µL of acridine orange were added and incubated for 1 hr. After the incubation, the cells were viewed under an epifluorescence microscope, in which the live cells emit green and the dead cells emit red.

Cytoprotective assay

The cytoprotective assay is the assay employed to assess cell viability using 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), which calorimetrically measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria, where it is reduced to an insoluble, dark purple formazan product. The toxicity to the cells was caused by the incubation of cells with gentamicin. The cells were then solubilized with an organic solvent (e.g., isopropanol), and the released, solubilized formazan reagent was measured spectrophotometrically.

RESULTS AND DISCUSSION

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF AQEOUS AND ETHANOLIC LEAF EXTRACT OF *MIMUSOPS ELENGI.*

A qualitative phytochemical examination was conducted on the aqueous and ethanolic leaf extract of *Mimusopselengi*to detect various phytochemicals, including carbohydrates, phenols, glycosides, flavonoids, quinines, steroids, tannins, saponins, terpenoids, and alkaloids. The findings of the phytochemical analysis are presented in Table 1. The aqueous extract of *Mimusops elengi* comprises carbohydrates, tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenols, and steroids. The aqueous extract did not contain terpenoid. The ethanolic leaf extract of *Mimusops elengi* contained carbohydrates, tannins, saponins, alkaloids, terpenoids, steroids, flavonoids, glycosides, quinine, and phenols.

S.NO	PHYTOCHEMICALS	AQUEOUS EXTRACT	ETHANOL EXTRACT
1.	Carbohydrates	+	+
2.	Tannins	+	+
3.	Saponins	+	+
4.	Alkaloids	+	+
5.	Flavonoids	+	+
6.	Glycosides	+	+
7.	Quinone	+	+
8.	Phenols	+	+
9.	Terpenoids	_	+
10.	Steroids	+	+

Table 1:Analysis of the phytochemicals present in the aqueous and ethanolic leaf extracts of *Mimusops elengi* was conducted.

ANTIBACTERIAL ACTIVITY

The antibacterial efficacy of the ethanolic leaf extract of *Mimusops elengi* was evaluated using the agar well diffusion method against four distinct types of bacteria, including two gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and two gram-negative bacteria (*Pseudomonas aeruginosa and Escherichia coli*). The efficacy of the ethanolic leaf extract was evaluated against four pathogenic bacteria at different doses (50, 100, and 150 µg/mL). The ethanolic leaf extract effectively inhibits the growth of all four bacterial strains. Nevertheless, the ethanolic leaf extract exhibits larger zones of inhibition that correspond to the concentration gradients. The zone of inhibition formed by the ethanolic leaf extract at three different concentrations is less than the zone formed by the positive control of Gentamicin in all four mediums containing microorganisms. Nevertheless, the ethanolic leaf extract has a substantial inhibitory zone, so effectively demonstrating its antibacterial action.

S.NO		DMSO 150 µg/mL	Gentamicin	Zone of inhibition		
				50 µg/mL	100 µg/mL	150 µg/mL
1.	Klebsiella pneumonia	-	25mm	17mm	18mm	19mm
2.	Pseudomonas aeruginosa	-	20mm	13mm	18mm	20mm
3.	Enterococcus faecalis	-	21mm	17mm	20mm	21mm
4.	Bacillus subtilis	-	20mm	14mm	16mm	18mm

Table:2 Ethanolic leaf extract of *Mimusops elengi* exhibits antibacterial action.

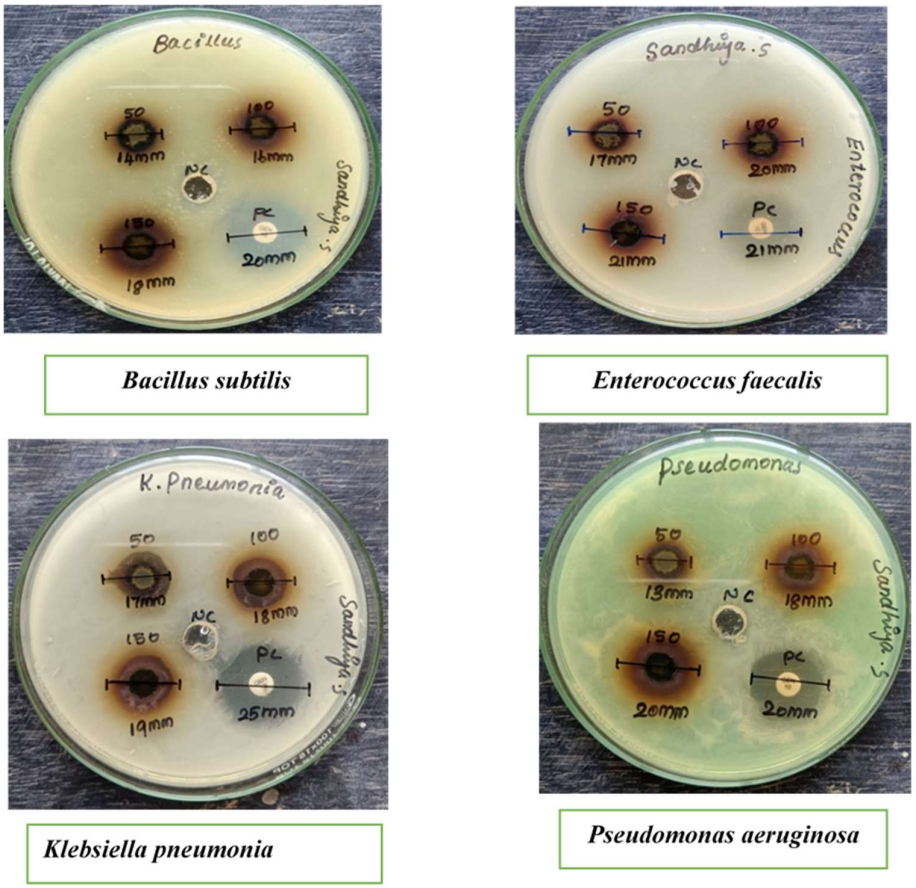


Figure:1 Antibacterial activity of ethanolic leaf extract of *Mimusops elengi*

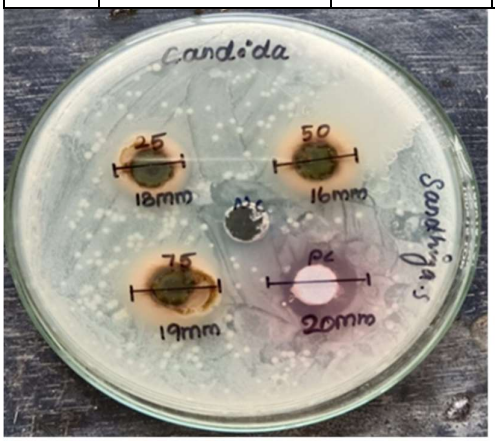
ANTIFUNGAL ACTIVITY

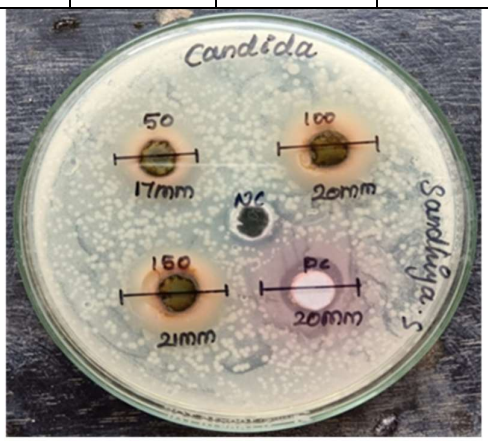
The fungicidal properties of *Mimusops elengi*were assessed by use SDA agar media to measure the extent of inhibition against Candida albicans at different concentrations (25, 50, 75 mg/mL) and (50, 100, 100 mg/mL). The ethanol-based leaf extract effectively inhibits the growth of Candida albicans. Nevertheless, an expanding area of inhibition becomes apparent as the concentration gradient increases. Therefore, the ethanolic leaf extract produces a substantial zone of inhibition.

Table:3 Antifungal activity by ethanolic extract of *Mimusops elengi*

S.NO	Organism	DMSO 75 µg/mL	Fluconazole	Zone of inhibition		
				25 µg/mL	50 µg/mL	75 µg/mL
1.	Candida albicans	-	20mm	13mm	16mm	19mm

S.NO	Organism	DMSO 150 µg/mL	Fluconazole	Zone of inhibition		
				50 µg/mL	100 µg/mL	150 µg/mL
1.	Candida albicans	-	20mm	17mm	21mm	20mm





Candida albicans

Figure:2Antifungal activity of ethanolic leaf extract of *Mimusopselengi*

IN VITRO ANTIOXIDANT ACTIVITY

The antioxidant activity of *Mimusops elengi* ethanolic leaf extract was assessed using the DPPH radical-saving test. The ethanol-based extract derived from the leaves of *Mimusops elengi* demonstrates the ability to scavenge DPPH radicals. The ethanolic leaf extract has a better value compared to ascorbic acid at initial doses of 10 and 20. Nevertheless, at a concentration of 30, the activity is almost identical to that of traditional ascorbic acid. Moreover, at doses of 40 and 50, the activity has a greater value compared to ascorbic acid. As the quantities of ascorbic acid and ethanolic leaf extract increase, the absorbance also increases, indicating that the potential of *Mimusops elengi* is diminished by free radicals.

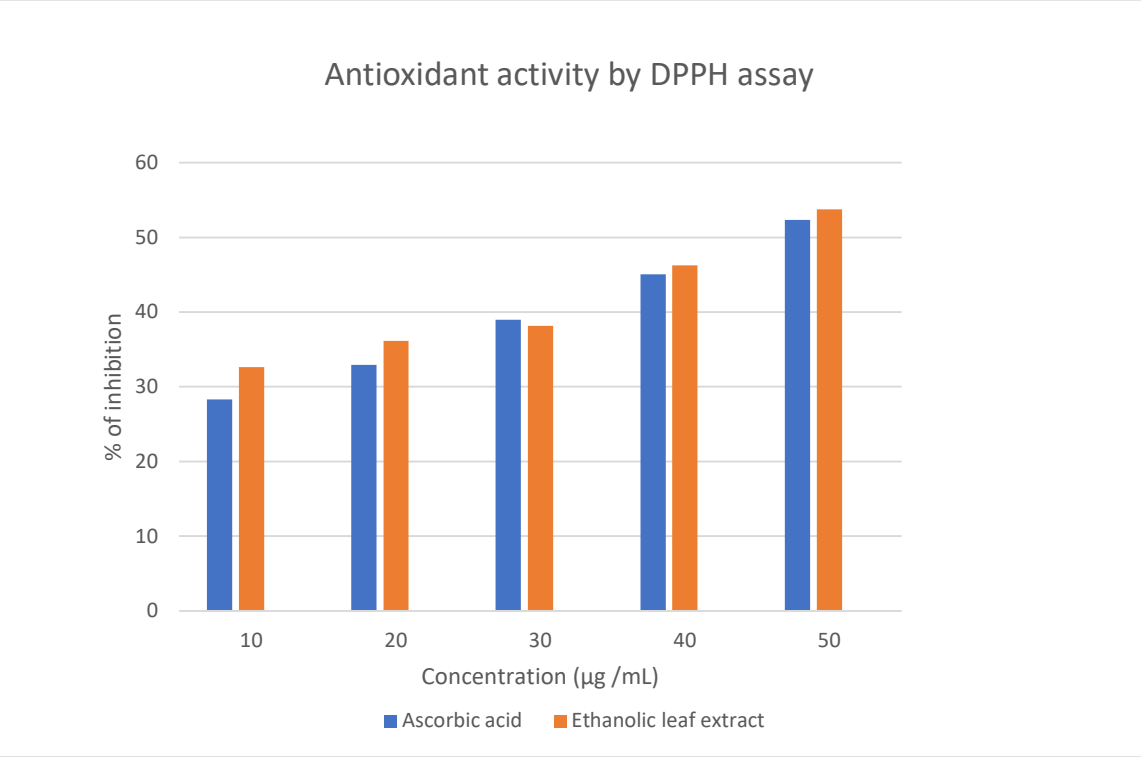


Figure 3: Antioxidant activity by DPPH assay

IN VITRO NEPHROPROTECTIVE ACTIVITY

Epifluorescence staining

The protective properties of the ethanolic leaf extract of "*Mimusops elengi*" against the harmful effects of gentamicin on cells were investigated using epifluorescence staining and a fluorescent microscope. The results of this analysis are displayed in Figure 5. The cellular changes induced by gentamicin include chromatin condensation, cell shrinkage, and other modifications, all of which are indicative of cellular fragmentation. Vitamin E, employed as a positive control, exhibited nuclei with a green hue, signifying its protective properties (Fig. 5a). Similarly, the application of ethanolic leaf extract led to the development of a vivid green

hue, accompanied by undamaged chromatin, as depicted in Figure 5b. The staining assay using fluorescence microscopy confirmed the cytoprotective properties of the plant extracts by detecting the presence of green-colored cells.

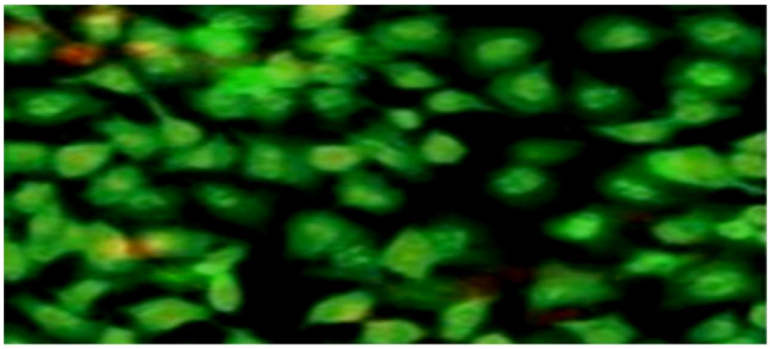


Fig. 5 a Control

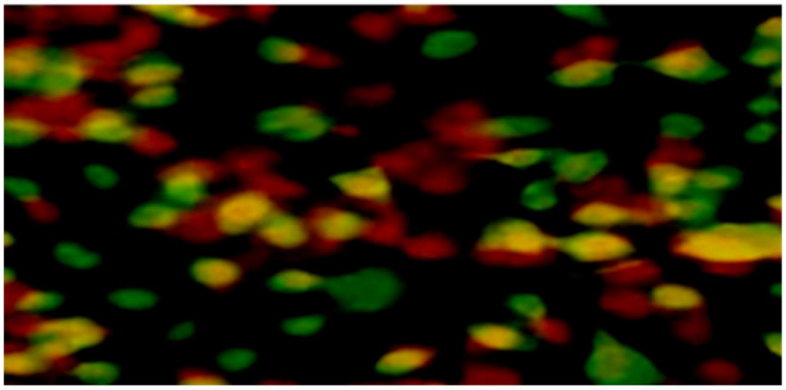


Fig. 5b Treated

Fig. 5: Epiflourescent staining of the ethanolic leaf extract of "*Mimusopselengi*(1a) live gentamicin-induced toxic HEK 293 cells after the treatment of cells with vitamin E, which is used as a positive control. (1 b) live gentamicin-induced toxic HEK 293 cells after the treatment of cells with ethanolic leaf extract of "*Mimusops elegans*"

Cytoprotective assay

The cytotoxicity of cells generated by toxins was evaluated using an MTT test to determine the effectiveness of the ethanolic leaf extract of "*Mimusops elengi*" in preserving cell viability. This viability test relies on the capacity of the mitochondrial succinate-tetrazolium reductase machinery to transform the yellow tetrazolium salt MTT into a purple formazon dye. This test is designed to evaluate the existence of viable cells following exposure to toxins. In this experiment, cellular toxicity was caused by the administration of gentamicin. Figure 10 illustrates the percentage of survivorship produced by the plant extracts. The lowering of MTT by the ethanolic leaf extract of "*Mimusops elengi*" (Fig. 10) resulted in the development of the formazon product. This formation was found to be dependent on the dosage

and was shown to indicate the presence of metabolically active cells. The cells were subjected to varying doses of the test substance for a duration of 48 hours, and the extracts were assessed for their cytotoxic effects. The HEK 293 Cells line reached a maximum capacity of 71.44% when treated with the highest dose of Ethanolic Leaf Extract from "*Mimusops elengi*". The concentrations of *Mimusops elengi* leaf extract utilized for treatment and their corresponding cell viability percentages are presented in Table 5 and illustrated in Figures 6 and 7.

Table.4 Assessment of the harmful effects of the ethanolic leaf extract of "*Mimusopselengi*" on the HEK293 cell line

S.No	Concentration (µg/ml)	OD Value	Cell viability (%)
1	1000	0.253	35.58
2	500	0.289	40.64
3	250	0.325	45.71
4	125	0.362	50.91
5	62.5	0.399	56.11
6	31.2	0.436	61.32
7	15.6	0.472	66.38
8	7.8	0.508	71.44
9	Cell control	0.711	100

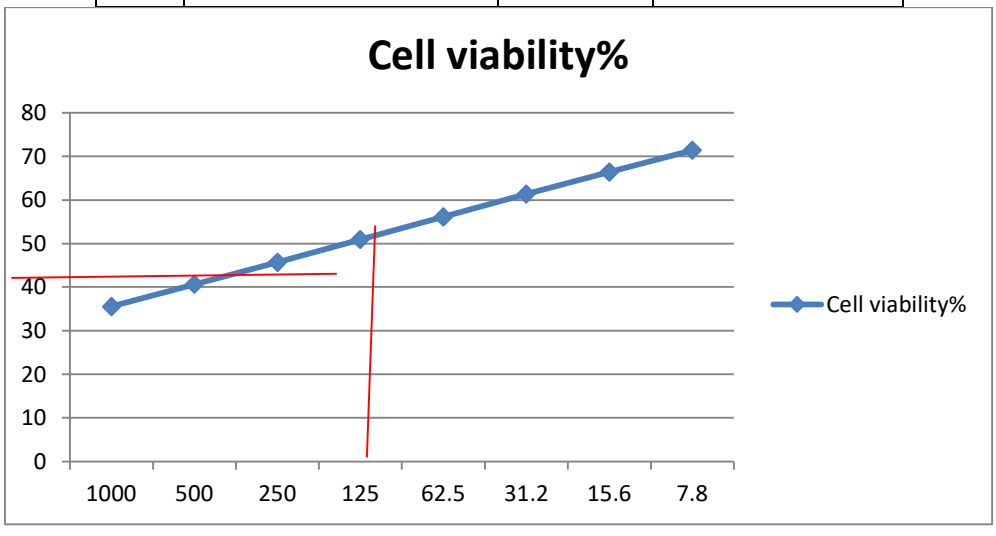


Figure 6: Assessment of the harmful effects of the ethanolic leaf extract of "*Mimusopselengi*" on the HEK293 cell line

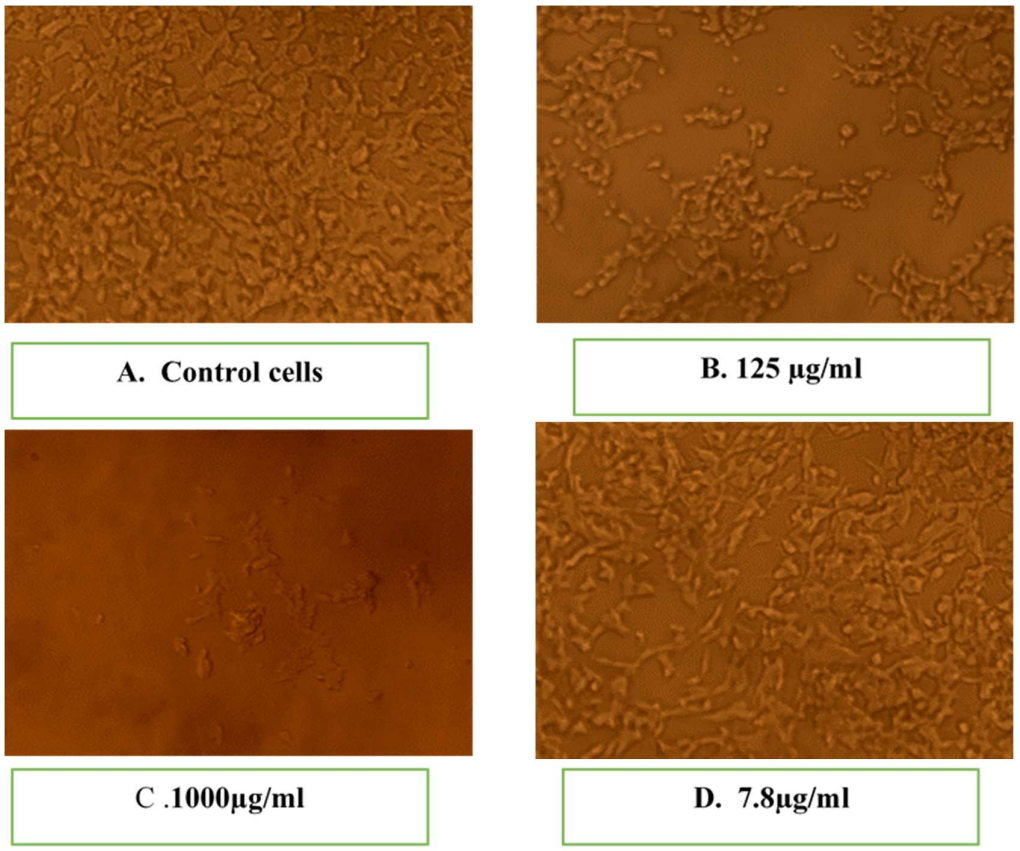


Figure7: Microscopic image demonstrating the in vitro curative properties of Ethanolic Leaf Extract of "*Mimusops elengi*" on: The HEK293 cell line was incubated in conditions with or without *Mimusops elengi* leaf extract and control; images were taken 24 hours later. (a) Control; (b) Treated 125 µg/ml into gentamicin-induced cell line; (c) Treated 1000 µg/ml into gentamicin-induced cell line; (d) Treated 7.8 µg/ml into gentamicin-induced cell line.

CONCLUSION

The present study aimed to assess the antioxidant, and nephroprotective properties of the ethanolic leaf extract of "*Mimusops elengi*". The ethanol extract of leaves, at a lower concentration, does not demonstrate toxicity towards the HEK293 cell line. This study highlights the potential of the ethanolic leaf extract of "*Mimusops elengi*" to function as an antioxidant, and nephroprotective agent under laboratory conditions. The medicinal effects of this substance may be attributed to the presence of phenolic compounds, specifically flavonoids and proanthocyanidins. Further investigation is necessary to evaluate the potential of this particular plant as a nephroprotective herbal medication.

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