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Phytochemical screening, antioxidant and cytotoxic activity of *Adansonia digitata* L., *Punica granatum* L., and *Mangifera indica* L. extracts prepared in different solvents against *Schizosaccharomyces pombe* cells

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Abstract
Plants and their bioactive compounds are in medicinal practices since ancient times. Several medicinal plant species and their phytochemicals inhibit the progression and development of cancer. The present study aims to evaluate antioxidant potential, cytotoxic activity and apoptosis inducing ability of selected plant extracts prepared using different solvents for extraction. The extracts of *Adansonia digitata* (leaves), *Punica granatum* (peels) and *Mangifera indica* (peels) were prepared using three different solvents Methanol, Methanol: water (7:3) and Hexane: water (7:3). Their phytochemical screening revealed the presence of various secondary metabolites. The methanol: water extract of *P. granatum* (peels), *A. digitata* (leaves) and methanolic extract of *M. indica* (peels) confirmed robust antioxidant activity through DPPH radical scavenging method with an IC_{50} value of 0.87µg/ml, 1.57µg/ml and 1.60µg/ml respectively. The methanolic extracts demonstrated substantial amount of cytotoxicity on *Schizosaccharomyces pombe* cells by affecting their growth and viability.The apoptosis inducing ability of plant extracts on yeast cells was checked through AO-EtBr dual staining. Further DNA isolation was carried out from yeast cells after 24h treatment of extracts showing high cytotoxicity and antioxidant activity. Results showed that control DNA was forming clear band while DNA of yeast cells treated with extract of *A. digitata* (leaves) (Methanol: Water), *P. granatum* (peels) (Methanol: Water) and *M. indica* (peels) (Methanol) were forming a smear indicated that extracts were altering the integrity of DNA. These plant extracts contain phytochemicals which are responsible for cytotoxic activity which may have potential to use as anticancer agents.

Keywords: Cold maceration method, phytochemical screening, cytotoxicity assays, antioxidant activity, *Schizosaccharomyces pombe*

1. Introduction
Medicinal plants are the reservoirs for novel chemical substances that provide benefits and serve as curative agents for various ailments. Plants-derived medicines are gaining prevalence in contemporary world as natural alternatives, since they possess pharmacological properties and have profound effect on prevention of diseases. The plants secondary metabolites are appreciated for their efficacy and lower toxicity as compared to synthetic drugs, since they are presently recognized as therapeutically useful molecules which can be potential alternatives for the development of anticancer drugs [Iqbal *et al*., 2017] [11]. Secondary metabolites like alkaloids, phenolic compounds, terpenoids, and sulphur-containing compounds have demonstrated significant therapeutic effects with a variety of chemical configurations [Fakhri *et al*., 2022] [8].

The present study was conducted to evaluate the potential of methanolic and hexane crude extracts of *Adansonia digitata* (leaves), *Punica granatum* (peels), and *Mangifera indica* (peels) for their antioxidant property, cytotoxic activity and apoptosis inducing potential on *S. pombe* cells.

The *Schizosaccharomyces pombe* is an excellent model species for studying genetic and biochemical investigations, which aids in understanding of basic cellular functions. Cell cycle regulation and many cellular mechanisms in fission yeast are identical to those found in humans. As a result, data from yeast research can be generalized to the human system [Thakor *et al*., 2016] [26]. Therefore, *S. pombe* cells were utilized as a model organism for this study to investigate the cytotoxicity of leaf/peel extracts.

The *Adansonia digitata*, also referred to as the Baobab, belongs to Malvaceae family. These are long-lived pachycauls with socioeconomic importance with a life span of approximately 2,000 years. *A. digitata* leaves are valued for their high level of mucilage (12%) and calcium, which improves easy meal absorption and controls intestinal transit. They are also utilized in rural regions to make cool drinks, offer minerals and vitamins, and are a key ingredient of sauces [Korbo *et al*., 2013] [15]. The leaves of *A. digitata* are used medicinally to treat conditions such as asthma, malaria, blood clearing, inflammation, kidney, and bladder problems, as well as to cure diarrhoea and fever [Kamatou *et al*., 2011] [13]. Thus, *A. digitata* (leaves) has been reported for Antiviral [Selvarani *et al*., 2009] [23], Anti-inflammatory [Ayele *et al*., 2013] [4], Antimicrobial and Antioxidant activities [Kabbashi *et al*., 2014] [12].

The *Punica granatum* is a member of the Punicaceae family. It is also known as pomegranate, grenade and granats. It has been widely utilized as a traditional medicine in many nations to treat dysentery, acidosis, diarrhoea, hemorrhage, and respiratory diseases. It is also said to have antioxidant, anti-atherosclerotic, and antiviral effects. Pomegranate peels have a high concentration of phenolic substances, including flavonoids (anthocyanins, catechins, and other complex flavonoids) and hydrolysable tannins (punicalin, pedunculagin, punicalagin, gallic, and ellagic acid) [Sabbah *et al*., 2017] [2]. The peels have been reported to exhibit Antiproliferative [Yassin *et al*., 2021] [29], Antimicrobial [Al-Zoreky *et al*., 2009] [3], Anticancer [Rani *et al*., 2016] [20], Antibacterial and Antifungal activities [Dahham *et al*., 2010] [6].

The *Mangifera indica* commonly referred to as mango is an important tree of family Anacardiaceae and is native to tropical Asia. Mango peel is a unique source of polyphenols, dietary fiber and carotenoids that have medicinal potential. The peels possess significant quantity of phytonutrients, carotenoids, polyphenols, vitamin E and C, along with antioxidant characteristics, due to which it has currently received a lot of significance in the scientific research [Ajila *et al*., 2007] [2]. The phytochemicals found in mango peel may provide protection against ROS-induced oxidative cell damage as a result of their antioxidant capabilities. Moreover, mango peels are a rich source of cellulose, hemicellulose, dietary fiber, lipids, enzymes, protein, and pectin [Sogi *et al*., 2013] [25]. This species is thought to have a wide variety of therapeutic applications, such as Antimicrobial [Thambi *et al*., 2016] [27], Antidiabetic [Mistry *et al*., 2023] [17], Immunostimulant and Anticancer potency [El-Hawary *et al*., 2014] [7].

2. Materials and Methods

2.1 Collection of plant parts and preparation of crude extracts

The plants were collected from nearby areas of Anand district, Gujarat, India, in December 2022. The gathered plant materials (leaves/peels) were washed under running tap water and allowed to air dry for 5-6 days at 25 °C. The dried leaves/peels were ground to make powder and stored in air-tight bags for further use.

The cold maceration method was used to prepare the plant's crude extracts. Ten grams of leaves/peels powder was soaked into 100ml of three different solvents [Methanol] and [Methanol: water (7:3) or Hexane: water (7:3)] and allowed to settle for 24 hours followed by filtration through Whatman filter paper no. 1. Filtrate was then poured into a bowl to allow the solvent to evaporate. After the solvent had completely evaporated, the remaining solvent extract was scraped off and redissolved into the same solvent as per the final weight of extract obtained. The extracts (Concentration: 100mg/ml) were filled into falcon tubes. The percentage yield was calculated as per the equation mentioned by Thakor *et al*., [2016] [26].

Percentage yield= (B-A)*100; where A=weight of dried powder of leaves/peels before extraction and B=weight of dried powder of leaves/peels after extraction.

2.2. Phytochemical investigation of plants crude extracts

Phytonutrients are the substances produced by plants that have health-promoting benefits to humans and are responsible to protect plants against infections.

The powder of *A. digitata* (leaves), *P. granatum* (peels), and *M. indica* (peels) extracted in different solvents were subjected to preliminary phytochemical analysis, to check the presence of various secondary metabolites like alkaloids, flavonoids, saponins, tannins, terpenoids, coumarins, phenols, polyphenols and cardiac glycosides. Phytochemical tests were performed according to the standard tests mentioned by Savithramma *et al*., [2011] [22] and Pandey *et al*., [2014] [19].

2.3 Antioxidant activity of crude extracts by DPPH Assay

The phytochemicals found in plants are responsible for expressing antioxidant capacity, which works to prevent oxidative damage induced by the production of ROS. It has been suggested that these phytochemicals may also reverse damage to cellular macromolecules leading to degenerative diseases which has a negative impact on human health.

The ability of the extract to scavenge DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical was determined according to the method described by Mensor *et al*., [2001] [6]. TheDPPH assay was performed by preparing 0.1 mM DPPH reagent in methanol, 1mg/ml of plants crude extracts as samples and Ascorbic acid (1mg/ml) as standard. For standard, series of aliquots of different concentrations were prepared (100, 200, 300, 1000 µg/ml) with the addition of methanol to make the volume of 1ml. For samples different concentrations of aliquots (10, 20, 30, 40 and 50 µg/ml) were prepared by adding the same solvent, in which plants crude extract were extracted (Methanol, Methanol: Water and Hexane: water), to the samples to make the volume to 1 ml. Then after, a similar method was followed for both the standard and samples. From each tube 500 µl of solution was transferred to individual amber tubes, to which 1500 µl of DPPH reagent was added and all the tubes were incubated in dark along with a control (DPPH reagent) for 30 minutes. After incubation, absorbance for standard and samples were read at 517 nm using UV-visible spectrophotometer and % inhibition was calculated using the following equation.

% Inhibition= Absorbance of control- Absorbance of sample/ Absorbance of control* 100.

The IC_{50} values for the standard and samples was estimated by plotting Concentration (µg/ml) versus % Inhibition graphs. The IC_{50} (Half maximal inhibitory concentration) refers to the quantitative measure of the potency of a substance in inhibiting a specific biological or biochemical function.

2.4 Culturing of *S. pombe* cells

For cytotoxicity experiments, *S. pombe* cells were grown on YPD (1% Yeast extract, 2% Peptone, 2% Dextrose and 2%

agar) agar media by four flame striking method and allowed to grow for 24hours at 30ºC in an incubator. After the growth of cells (approximately 80% confluency), the YPD plates were kept in the refrigerator and were revived on alternate days to maintain purity of cells cultured.

2.4.1 Determination of cytotoxicity on *S. pombe* cells by trypan blue assay

The amount of viable and non-viable cells in a culture that has been treated with plants crude extract was determined with the aid of the cytotoxic experiment.

A colony of *Schizosaccharomyces pombe* was inoculated into 50 ml of YEG broth (0.25 gm of Yeast extract and 1.5 gm of Glucose into 50 ml of distilled water) and incubated for 24 hours at 37 °C. Cell growth was measured using a spectrophotometer at 600 nm after incubation. After reaching 1.5-2*10^6 cells/ml, the cells were treated with 500 µl of plant extracts (concentration: 100 mg/ml) in 50 ml of broth flask and one of the broth flasks was left untreated (Control). The culture was incubated at 37 °C for another 24 hours under shaking conditions. Following the incubation period, 10ml of culture were transferred from each flask to a 15ml centrifuge tube and centrifuged at 8,000 rpm for 10 minutes. The supernatant was removed, and the pellets were washed twice with Phosphate Buffered Saline (PBS) [0.2 gm KCL, 8 gm Nacl, 1.44 gm Na_2HPO_4, 0.245 gm KH_2PO_4 (pH 7.4)]. Followed by centrifugation and dissolving pellets in 1 ml of PBS. From the suspension, 80µl of treated cells and 20 µl of trypan blue dye (0.4%) was taken in amber microcentrifuge tubes and incubated for 10minutes at room temperature. About 10 µl of cells suspension was placed on microscope slide and observed under 40x. The viable (unstained) and non-viable (stained) cells were calculated manually and the viability was determined according to the formula mentioned by Coligan *et al*., [2001] [5].

Viability of cells (%) = No. of live cells observed / Total no. of cells × 100

2.4.2 Detection of apoptosis inducing property of *S. pombe* cells treated with plant extracts

Co-staining of cells with Acridine Orange/Ethidium Bromide (AO/EtBr) provides information about apoptotic body formation and nuclear changes that are features of apoptosis [Kasibhatla *et al*., 2006] [14].

A similar protocol for growth, treatment and suspension preparation of *S. pombe* cells was followed as mentioned above for cytotoxicity assay.About, 10 µl of cell suspension with 5 µl of 1:1 Ao/EtBr solution [Acridine orange (100 µg/ml) and Ethidium bromide (100 µg/ml) in PBS] was incubated at room temperature for 10mins. Then, 10 µl of cells suspension was placed on microscope slide. The cells were observed under fluorescence microscope using 40x objective lens.

The apoptotic morphology was evaluated using Acridine orange (AO) and Ethidium Bromide (EtBr) co-staining. Acridine orange is hydrophobic, which enables it to pass through the cell membrane quickly and enters the cytoplasm. Acridine orange when binds to single stranded DNA or RNA, red fluorescence is emitted whereas green fluorescence is released when bound to double stranded DNA. The Ethidium bromide is impermeable to healthy cell membranes and intercalate the DNA and produces red or orange fluorescence when cells lose their membrane integrity [Ude *et al*., 2022] [28]. Thus, Ao/EtBr staining was done to evaluate the potential of plants extract to induce apoptosis in treated *Schizosaccharomyces pombe* cells.

2.4.3 DNA isolation of treated *Schizosaccharomyces pombe* cells and analyzing DNA integrity on agarose gel electrophoresis

The plants crude extracts of *P. granatum* (leaves) (Methanol: Water), *A. digitata* (leaves) (Methanol: Water) and *M. indica* (peels) (Methanol) revealed considerable level of cytotoxicity on *S. pombe* cells as well as antioxidant potential. To confirm whether the treated cells undergoing apoptosis also affected the integrity of macromolecules, therefore, the DNA of *S. pombe* cells was isolated and their integrity was examined on 1% agarose gel electrophoresis under UV-transilluminator.

Isolation was carried out according to the Hoffman *et al*., [1987] [10] with minor modifications. A colony of cells *S. pombe* from YPD agar plate was inoculated into broth tubes and kept in shaking incubator at 30 ºC. After 24hours, when the growth had reached to approximately 1.5-2*10^6 cells/ml, the cells were treated with plant extract (100 µl/10 ml of broth), and one of the tubes without any extract treatment was kept as control. Again, the broth was kept in shaking incubator at 30 ºC. After 24 hours, about 10ml of cell cultured broth was taken into 15 ml of centrifuge tubes and centrifugated at 8,000 rpm for 10minutes. The supernatant was discarded and pellets were washed twice with 1x PBS (1-2 ml). Again, the supernatant was discarded, pellets resuspended into 1ml of distilled water and centrifuged at 8000 rpm for 10 mins. 1ml of lysis buffer [volume 500ml: 100mM NaCl (2.74g), 10mM Tris buffer (2.88 g), Triton-X 100 (10 ml), 1mM EDTA (0.186 g) and SDS (1 g)] was added to all the tubes followed by addition of chilled Phenol: Chloroform: Isoamyl alcohol (25: 24: 1) separately (1ml: 0.8 ml: 0.2 ml), along with acid washed glass beads. Each tube was by kept in ice for 1min and 1min vortex (same was repeated for 10 mins). Immediately the tubes were centrifuged at 10,000 rpm for 10min at 4 ºC. The upper layer was transferred into separate centrifuge tubes, to which 1/10th volume of 3 Molar Sodium acetate (pH 5.2) and 2.5x volume of chilled 100% ethanol was added. Followed by centrifugation at 8,000 rpm for 20 mins. The pellets were washed with ice-chilled 70% ethanol (1 ml) and centrifuged at 8,000 rpm for 20 mins. Then the supernatant was removed, pellets were air-dried to evaporate ethanol completely and 30-40 µl of Tris-EDTA (pH 7.5) was added. Thus, the integrity of DNA of *S. pombe* cells was analyzed when separated on 1% agarose gel electrophoresis. The bands were visualized in UV-transilluminator and the DNA integrity of untreated and treated *S. pombe* cells were examined.

3. Results and Discussion

3.1 Percentage yield of the extracts

In order to determine which solvent gives better yield along with the higher number of phytochemicals, three distinct solvents were used in this study. The percent yield of plant extracts (dry weight) obtained using three different solvents, turned out to be highest in methanol, followed by methanol: water (7:3) and lowest in hexane: water (7:3).The methanol extracts gave the highest yield being highest polar solvent while hexane being the lowest polar solvent gave the least percentage recovery.The results (Table 1) demonstrated that methanol works best as an extraction solvent for the extraction of several active phytochemicals.

Table 1: Percentage yield of plant extracts in different solvents

Part of plants	Solvents		
	Methanol	Methanol: Water	Hexane: Water
A. digitata (leaves)	14%	16.7%	4%
P. granatum (peels)	33.5%	39%	7%
M. indica (peels)	33.08%	9.05%	0.18%

3.2 Phytochemical screening

The phytochemical analysis of plant crude extracts in various solvents revealed the presence of numerous secondary metabolites such as alkaloids, tannis, terpenoids, flavonoids, coumarins, steroids, cardiac glycosides, emodins, phenols, polyphenols and saponins. The solvent that produced the highest yield revealed the presence of a greater number of secondary metabolites, i.e., methanol, compared to methanol: water and hexane: water. The detailed results of phytochemicals present in plant extracts with respect to solvents in which they were prepared are presented in Table 2.

In present study, presence of flavonoids and saponins along with other secondary metabolites was observed in *A. digitata* leaves (Methanol extract) while, Oloyede *et al*., [2010] [18] reported absence of flavonoids and saponins in similar extract.

The Phytochemical screening of *P. granatum* peels extract in methanol showed the presence of alkaloids however, research by Sabbah *et al*., [2017] [2] showed the absence of alkaloids, saponins and presence of steroids, flavonoids, phenols and cardiac glycosides.

The current analysis discovered the absence of terpenoids and presence of cardiac glycosides in methanol and methanol: water extracts of *M. indica* peel extract. Whereas, according to the report of Falusi *et al*., [2017] [9] the results of qualitative phytochemical screening of methanolic extracts of ripe and unripe mango peels revealed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids and phenolic compounds in both ripe and unripe mango peels extracts while glycosides were not detected in both extracts.

Table 2: Phytochemical tests of plants extracts prepared in different solvents

Secondary Metabolites	*Adansonia digitata* (leaves)			*Punica granatum* (peels)			*Mangifera indica* (peels)		
	Methanol	Methanol: Water	Hexane: Water	Methanol	Methanol: Water	Hexane: Water	Methanol	Methanol: Water	Hexane: Water
Alkaloids	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
Tannins	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve
Flavonoids	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
Terpenoids	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve
Coumarins	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
Steroids	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve
Cardiac glycosides	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
Emodin	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
Phenols	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Polyphenols	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
Saponins	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve

Abbreviations: +ve (present), -ve (absent)

3.3 Antioxidant activity of plant extracts

The graph of standard ascorbic acid's concentration verses % inhibition was plotted (Figure: 1). Similar graphs were prepared for each plant extracts from which IC_{50} concentration was calculated (Table 3). The IC_{50} of Ascorbic acid standard was found to be 1406.3 µg/ml.

Table 3: IC_{50} (µg/ml) values of plants crude extracts in three solvents

IC_{50} (µg/ml) values of plants crude extracts			
Part of plants	Solvents		
	Methanol	Methanol: Water	Hexane: Water
A. digitata (leaves)	2.20	1.57	16.23
P. granatum (peels)	4.93	0.87	23.06
M. indica (peels)	1.60	1.21	29.32

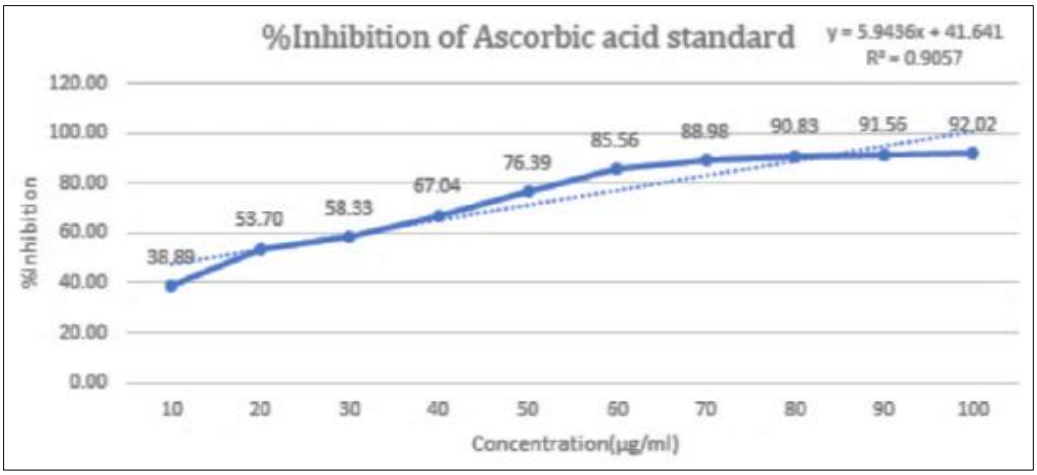

Fig 1: % Inhibition of Ascorbic acid standard

The plant extracts of *P. granatum* (peels) (Methanol: Water), *A. digitata* (leaves) (Methanol: Water) and *M. indica* (peels) (Methanol) exhibited better antioxidant activity among all the extracts with their IC_{50} as 0.87µg/ml, 1.57µg/ml and 1.60 µg/ml respectively. The results revealed that crude extracts were able to scavenge free radicals at very low concentrations in µg.

3.4 Cytotoxic activity of plant extracts on *S. pombe* cells

The dye exclusion test was performed to determine the number of viable or non-viable cells present in a cell suspension after the 24-hour treatment of plant extracts. Trypan blue is a non-permeable cell membrane dye which is derived from toluidine. The dye enters cells with impaired cell integrity and stains the cytoplasm blue, making it simpler to distinguish them as dead or non-viable cells. Cells with intact membranes, however, continue to be colourless even after treatment, making them easy to distinguish as living or viable cells. The photomicrographs (Figure 2 to 5) are of trypan blue dye exclusion test after the treatment of plants crude extracts, extracted in different solvents. Live and dead cells were calculated manually and % cytotoxicity (Table: 4) was determined.

Table 4: % Cytotoxicty of plant crude extracts in different solvents

Crude Extracts	% Cytotoxicity		
	Solvents		
	Methanol	Methanol: water	Hexane: Water
A. digitata (leaves)	36.40%	48%	41.48%
P. granatum (peels)	26.28%	51.52%	42.75%
M. indica (peels)	45%	33.63%	28.98%

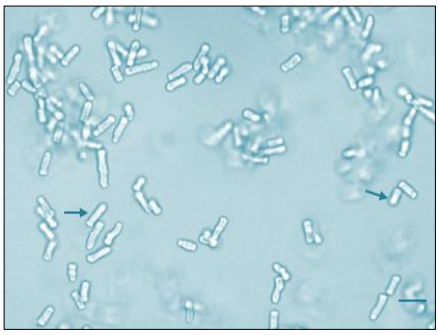

Fig 2: Trypan blue staining on *S. pombe* cells (control). Arrows indicate the *S. pombe* cells with intact cell membrane and colourless cytoplasm

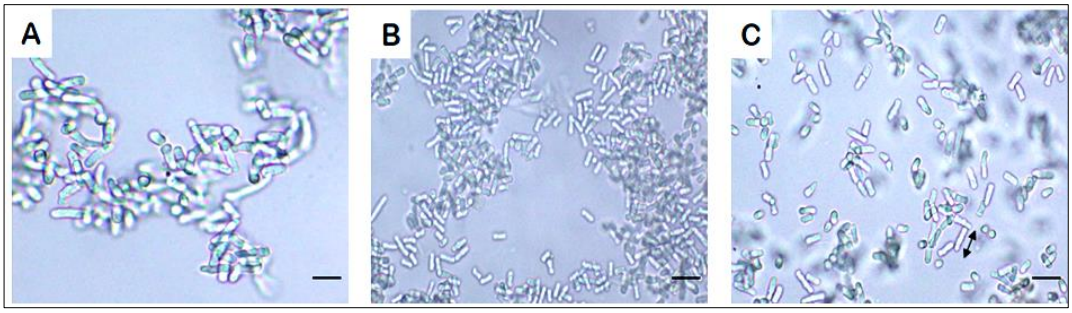

Fig 3: Trypan blue staining on *S. pombe* cells treated with plants extract in Methanol. A- *A. digitata* (leaves), B- *P. granatum* (peels), C- *M. indica* (peels)

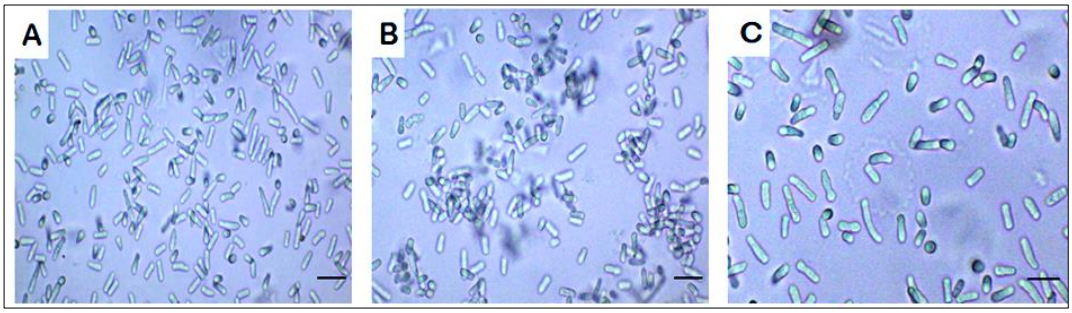

Fig 4: Trypan blue staining on *S. pombe* cells treated with plants extract in Methanol: Water. A- *A. digitata* (leaves), B- *P. granatum* (peels), C- *M. indica* (peels)

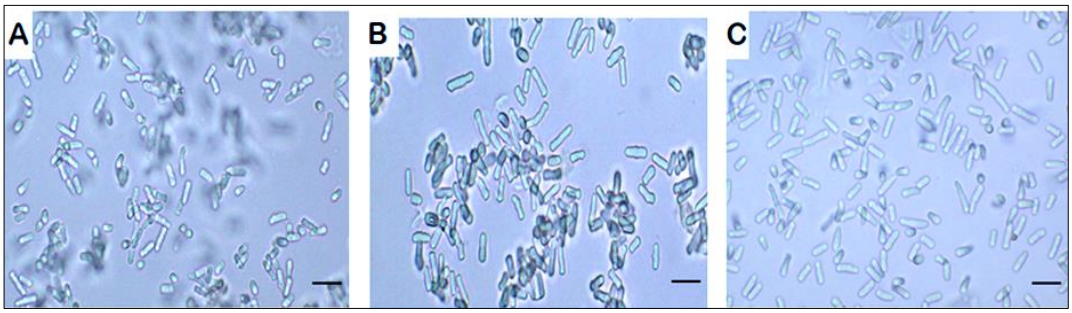

Fig 5: Trypan blue staining on *S. pombe* cells treated with plants extract in Hexane: Water. A- *A. digitata* (leaves), B- *P. granatum* (peels), C- *M. indica* (peels)

The highest % cytotoxicity was observed in *P. granatum* (peels) extract (Methanol: Water) (51.52%) (Figure: 4B) followed by *A. digitata* (leaves) extract (Methanol: Water) (41.48%) (Figure: 4A) and *M. indica* (peels) extract (Methanol) (41%) (Figure: 3A).

The *M. indica* peels had the lowest levels of cytotoxicity, which were 28.98% in Hexane: water extract (Figure: 5C) and 33.63% in Methanol: water extract (Figure: 4C). The possible cause of reduced cytotoxicity could be appearance of less phytochemicals in Hexane: water extract as well as decreased ability to scavenge free radicals. Thus, higher percentages of cytotoxicity were displayed by the extracts with stronger antioxidant activity.

3.5 AO/EtBr staining

The apoptotic morphology was evaluated using Acridine Orange (AO) and Ethidium Bromide (EtBr) co-staining. It was done to evaluate the potential of plant extracts to induce apoptosis in treated *S. pombe* cells. Acridine orange stained both live and dead cells, whereas the Ethidium bromide stained those cells that had lost membrane integrity. The live cells appeared uniformly green color while the early apoptotic cells stained green and contained bright green dots in the nuclei as consequence of chromatin condensation and nuclear fragmentation. Late apoptotic cells incorporated ethidium bromide and therefore stained orange.

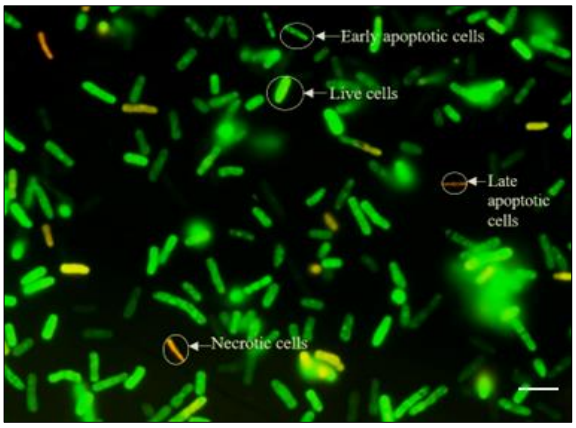

Fig 6: *S. pombe*cells after extract treatment in different apoptotic phases when stained with Ao/EtBr

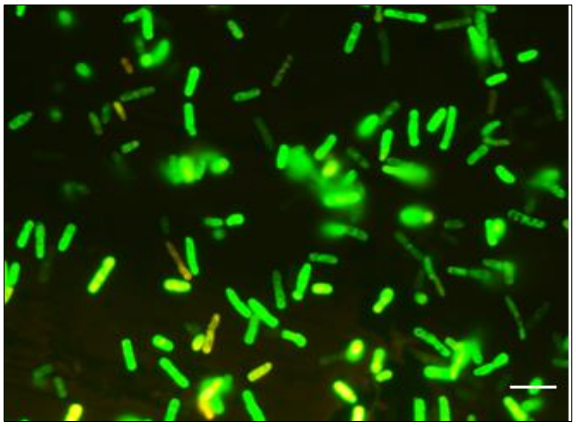

Fig 7: *S. pombe* cells (control) stained with Ao/EtBr

Observation revealed that higher proportion of late apoptotic cells which are stained orange with fragmented nuclei were present in the cells treated with crude plant extracts of *P. granatum* (peels) (Methanol: Water) (Figure: 9B) followed by *A. digitata* (leaves) (Methanol: Water) (Figure: 9A) and *M. indica* (peels) (Methanol) (Figure: 8C). Whereas untreated cells showed uniformly stained green cytoplasm and intact chromatin without fragmentation (Figure: 7).Cells treated with Hexane: Water extracts (Figure: 10) were found to have a greater number of live cells compared to cells treated with other extracts. Therefore, the extracts with significant cytotoxicity and antioxidant activity caused yeast cells to undergo apoptosis.

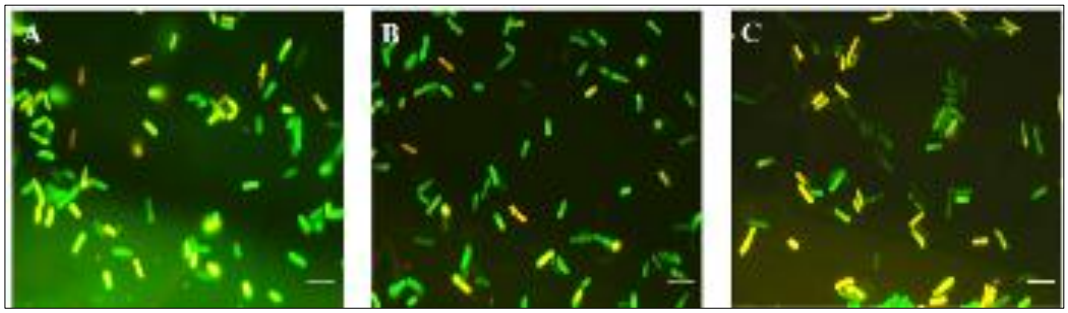

Fig 8: AoEtBr staining of *S. pombe* treated with plants crude extracts in Methanol A- *A. digitata* (leaves), B- *P. granatum* (peels), C- *M. indica* (peels)

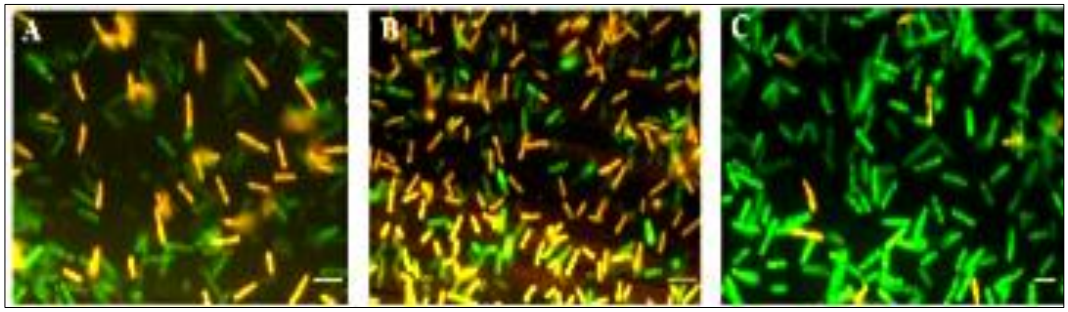

Fig 9: AoEtBr staining of *S. pombe* treated with plants crude extracts in Methanol: Water A- *A. digitata* (leaves), B- *P. granatum* (peels), C- *M. indica* (peels)

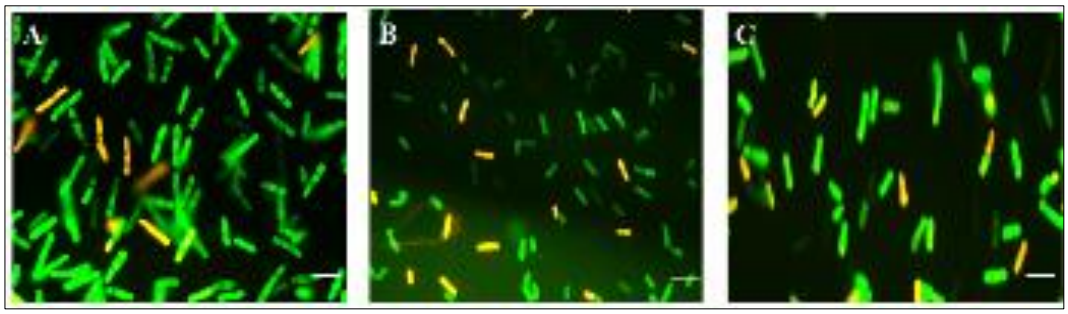

Fig 10: AoEtBr staining of *S. pombe* treated with plants crude extracts in Hexane: Water A- *A. digitata* (leaves), B- *P. granatum* (peels), C- *M. indica* (peels)

3.6 DNA isolation of yeast (*S. pombe*) cells treated with plant extracts

Further investigation was done on the extracts that exhibited high levels of cytotoxicity and antioxidant activity to check their effect on DNA integrity of *S. pombe* cells.

After 24 h treatment of *A. digitata* (leaves) extract (Methanol: Water), *P. granatum* (peels) extract (Methanol: Water) and *M. indica* (peels) extract (Methanol), *S. pombe* cells were subjected for DNA isolation. DNA from *S. pombe* cells treated with plant crude extracts formed smear when separated in 1% agarose gel electrophoresis, while the DNA from untreated cells appeared to be intact and represented by a single band (Figure: 11).Observing DNA band pattern on gel, it became apparent that plant extracts were able to alter the integrity of DNA compared to DNA of untreated cells.

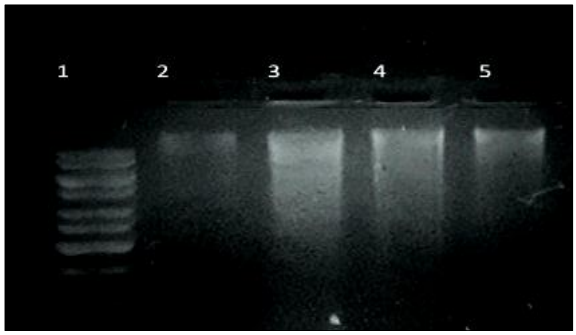

Fig 11: Agarose gel electrophoresis of Control and Treated *S. pombe* DNA. Lane 1: Ladder, Lane 2: Control, Lane 3: *S. pombe* DNA treated with *P. granatum* (peels) (Methanol: Water) extract, Lane 4: *S. pombe* DNA treated with *A. digitata* (leaves) (Methanol: Water) extract, Lane 5: *S. pombe* DNA treated with *M. indica* (peels) (Methanol)

As the mutagenic effects of phytochemicals are well-known and they may be one of the factors impacting the DNA integrity of *S. pombe* cells. The extracts exhibited considerable antioxidant capacity, suggesting that the production of ROS may have an impact on DNA integrity.

4. Conclusion

Plants are the reservoir of various chemical constituents and contain different biological and pharmacological properties. These findings disclosed that the methanol extracts gave the best extraction yield compared to other two solvents used. Phytochemical analysis showed rich contain of bioactive molecules in methanolic extracts and those phytochemicals might serve an important role in future to overcome the life-threatening illness. According to various research findings, phytochemical constituents and their derived analogues are the most promising choice for better and less toxic cancer treatment, as they are selective in their functions and acts specifically on tumor cells without affecting normal cells [Iqbal *et al*., 2017] [11] [Singh *et al*., 2016].

The Methanol: water extract of *P. granatum* (peels) had the highest DPPH radical scavenging activity at low concentrations, followed by *A. digitata* (leaves) in methanol: water and *M. indica* (peels) in methanolic extract. The cells treated with the same extracts revealed high cytotoxic activity and a greater proportion of cells undergoing early apoptotic and necrotic phase. In contrast, a greater number of live and early apoptotic cells were seen in the cultures treated with Hexane: water extracts.The treatment of plant extracts altered the DNA integrity of cells, which suggested that these crude extracts have a promising anti-cancer potential. Thus, these plant extracts contain some important phytochemicals which are responsible for high antioxidant capacity, cytotoxicity and apoptosis inducing potential. Further study is required to find out the responsible phytochemical/s that can be used in treatment of disease like cancer.

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