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Anticancer Potential and Antioxidant Properties of Artocarpus Heterophyllus (Jackfruit) Seeds

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A B S T R A C T

This research investigates the potential pharmaceutical and health-related applications of Artocarpus heterophyllus (Jackfruit) seed extracts. Using a combined approach of molecular docking simulations and experimental assays, the study examines binding potential, antioxidant activity, and cytotoxic effects of the extracts. The docking protocol's successful validation ensures reliability. Kaempferol D demonstrates high docking scores, indicating potential as a human tubulin inhibitor. Analysis of phenolic and flavonoid content reveals their presence, especially in the n-hexane extract. These compounds possess antioxidant properties, suggesting the extracts as natural antioxidant sources. DPPH radical scavenging assay confirms antioxidant potential in both ethanol and n-hexane extracts. Cytotoxicity assessment via the MTT assay highlights the n-hexane extract's anti-proliferative effects on U87 (MG) cells. This study underscores Artocarpus heterophyllus seed qualities and their potential in health and medicine, proposing them as a source for innovative anticancer therapies and combating oxidative stress-related ailments. Utilizing these seeds in diets or therapeutic treatments could advance health promotion and disease prevention strategies.

Keywords: *Artocarpus heterophyllus*, Molecular docking simulations, DPPH, Antioxidant activity, Cytotoxic effects, MTT assay, Anticancer therapies

1. INTRODUCTION

Jackfruit, native to the lush rainforests of the Western Ghats in India, has become a staple in South and Southeast Asia. Tripura, Assam, and West Bengal lead jackfruit production in India, with Tripura being the top contributor, offering jackfruits year-round. Apart from its culinary popularity, the jackfruit tree provides durable timber, fodder for livestock, and various medicinal properties attributed to its bark, roots, seeds, leaves, and fruit [Elevitch and Manner, 2006] [Das and Saha, 2020].

The Artocarpus genus, to which jackfruit belongs, presents a treasure trove of health benefits. Phytochemicals isolated from Artocarpus heterophyllus seeds demonstrate anti-inflammatory, anticancer, chemopreventive, and antioxidant properties [Morrison et al., 2021]. Compounds like artocarpin, isoartocarpin, and norartocarpetin offer a diverse array of therapeutic benefits, aiding in the treatment of fever, wounds, skin diseases, and even serving as remedies for snake bites [Prakash et al., 2009].

Notably, jackfruit seeds, often overlooked, boast a range of nutrients and potential health benefits [Hossain et al., 2014]. Rich in protein, fiber, healthy fats, and essential vitamins and minerals, they have antimicrobial, antioxidant, anti-inflammatory, and anticancer properties [Shanmugapriya et al., 2021]. Traditionally used in treating ailments like diarrhea and asthma, recent studies suggest their potential in reducing the risk of chronic diseases such as cancer, diabetes, and heart disease [Devi et al., 2021] [Burci et al., 2021].

With cancer being a global health challenge, the search for effective and safer anticancer medications is critical [Gupta et al., 2010]. Jackfruit seeds, containing bioactive compounds like phenolic compounds, flavonoids, and saponins, exhibit antioxidant activity, cytotoxic effects on cancer cells, and antiproliferative properties [Silva et al., 2019] [Burci et al., 2019]. This makes them a promising source for developing innovative anticancer therapies.

In the context of current research objectives, this study aims to explore the binding potential of chemical constituents from Artocarpus heterophyllus seeds against human tubulin, investigate the phytochemical composition, examine antioxidant properties, and explore the potential effect of extracts derived from jackfruit seeds on cancer cell lines proliferation [Morrison et al., 2021]. This research holds promise in uncovering valuable insights for cancer prevention and treatment strategies.

2. METHODS AND MATERIAL

Reagents:

All solvents and chemicals were of analytical grade and obtained from local suppliers. Methanol, dimethyl Sulfoxide (DMSO), polisorbate, formaldehyde, sodium chloride (NaCl), potassium chloride (KCL), & calcium chloride ($CaCl_2$), ascorbic acid, aluminium trichloride ($AlC1_3$), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ferric chloride, Folin-Ciocalteu reagent, potassium phosphate (monobasic and dibasic), sodium carbonate, MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5- diphenyltetrazolium Bromide), Ethanol, n-Hexane, etc.

2.1 Extraction procedure:

Seeds of Artocarpus heterophyllus (Jack fruit) were collected from local market washed with natural water twice and the white arils (seed coat) were pulled off & dried in Incubator at 40°C for 3-4 days without the removal of brown spermoderm. The dry Seeds Samples (79g) were grind using a grinder, then take the powder for powdered Samples.

The extraction process involved maceration of 25 grams of powdered samples in separate beakers containing 100 ml of a mixture of 80% ethanol and absolute n-hexane. The maceration was carried out with intermittent shaking for a duration of five days at room temperature. Afterward, the extracts were filtered using Whatman Grade I filter paper and concentrated using a rotary evaporator. The resulting concentrated extracts were stored in a refrigerator for future use [Eve *et. al*., 2020.,].

Figure 1. jackfruit seeds, chopped seeds, (Dry chopped seeds after 5 days, Dried seed extract powder, put sample in two different beakers having n-hexane and ethanol for extraction after 7 days, Filtration process after 12 days, Put sample in BOD incubator on 12 days to evaporate the water content, Use Rotatory evaporator after 2 weeks to make extract more condensed and to evaporate all water content.

2.2 Molecular docking studies:

Study Area:

The aforementioned research was conducted at Meerut Institute of Engineering and Technology in Meerut, India.

Methodology:

The 3-D structures of all the compounds were determined using Cresset's Flare 7.0. These manufactured structures underwent thorough optimisations. The wave function's spin state was changed to the singlet while the minimization was being done, and conventional SCF convergence was utilised for optimisation. All other parameters were kept at their usual settings.

Enzyme Used:

In this study, enzymes were employed, specifically those from PDB ISAO. The 3-D structures of proteins from ISAO and the human receptor (PDB ID ISAO) were obtained from the Protein Data Bank (https://www.rcsb.org). To isolate specific components, Flare 7.0 was utilized to extract water molecules, ions, and ligands from the protein molecule.

Protein Preparation Wizard:

To facilitate the docking analysis, the protein structure was prepared using the Protein Preparation Wizard. This involved assigning the correct bond orders and adding hydrogen atoms to the protein. Subsequently, an imperf usefulness algorithm was employed to perform a restrained minimization of the average Root-Mean-Square-Deviation (RMSD) of the hydrogen atoms, while keeping the heavy atoms unchanged.

Docking:

To facilitate the docking process, an interaction grid was generated for the protein structure. Specifically, in the active site of chain A, a grid was created for Tubulin protein (ISA0). The receptor grid for the PDB protein (ISA0) was developed using the bound inhibitor DAMA Colchicine as a reference structure to define the active site. The grid box was determined based on the bound ligand present in the protein structure, which was selected from the workspace. The extracted ligand underwent necessary corrections and was re-docked using the aforementioned grid parameters. Subsequently, the Root-Mean-Square-Deviation (RMSD) was calculated. Following the validation of the docking protocol defined by [Chaudhary et. al.,2022] a collection of prepared ligands was docked into the active site.

2.3 Total phenolic content

The modified Folin-Ciocalteu reagent method was used to determine the total phenol content. In this method, 1 mg of each crude extract was dissolved in 1 mL of methanol. A 10% Folin-Ciocalteu reagent solution was prepared by mixing 10 mL of the reagent with 90 mL of water. Additionally, a 5% Na_2Co_3 solution was prepared by dissolving 3 g of Na_2Co3 in 50 mL of water.

To perform the assay, 200 µL of each crude sample was taken in a test tube and mixed with 1.5ml of the 10% Folin-Ciocalteu reagent solution. The test tubes were then placed in a dark location for 5 minutes. Afterward, 1.5 mL of the 5% Na_2Co_3 solution was added to each tube, and the solutions were thoroughly mixed by hand. The test tubes were once again kept in the dark for 2 hours. Finally, the absorbance of each solution was measured using a UV-spectrophotometer at a constant wavelength of 750 nm, according to the references by [Shanmugupriya et. al.,2011.].

A Gallic acid calibration crude was created using a modified Folin-Ciocalteu reagent method. Initially, 3 mg of Gallic acid was dissolved in 10 mL of methanol, resulting in a concentration of 300 mg/L. This solution was then diluted by adding methanol to generate a series of standard concentrations: 200, 100, 50, and 25 mg/L. The same procedure was followed for the Gallic acid standard (Fig.9).

To determine the concentration, the absorbance of all standard solutions was measured using a UV-spectrophotometer at a specific wavelength of 750 nm.

Figure **2:** Folin-Ciocalteu reag ent method used to determine the total phenol content

2.4 Total flavanoid content

The concentration of flavonoids in milligrams per millilitre (mg/mL) was determined using the aluminium chloride ($AIC1_3$) method as described by [SHANMUGAPRIYA et. al.,2011.]. To perform the assay, 0.5 mL of the seed extract was mixed with 0.5 mL of distilled water and 0.3 mL of 5% $NaNo_2$ (fig.10). The mixture was then incubated at 25°C for 5 minutes. Next, 0.3 mL of 10% $AlC1_3$ was added immediately, followed by the addition of 2 mL of 1M NaOH. The absorbance of the reaction mixture was measured at 510 nm using a spectrophotometer. Quercetin was used as a standard for comparison.

Figure **3** Qurecetin standard test of flavonoid

2.5 Determination of DPPH radical scavenging activity

The DPPH scavenging activity was measured using a spectrophotometric method based on the principle that antioxidants can donate protons to scavenge the DPPH radical, leading to the formation of reduced DPPH. The reduction of DPPH results in a loss of colour, which can be quantified by measuring the decrease in absorbance at 517 nm [Devi et. al.,2021.].

To conduct the assay, extracts of different concentrations ranging from 10 to 50 µg/ml were prepared. The tubes containing the extracts were then made up to a total volume of 2ml with aqueous ethanol. A fresh solution of DPPH in aqueous ethanol at a concentration of 0.6mM was prepared.

Next, 0.5 ml of the DPPH solution was added to all the tubes containing the extracts. The contents of the tubes were mixed thoroughly and incubated in the dark for 30 minutes at room temperature. After the incubation period, the absorbance of each sample was measured at 517 nm using a spectrophotometer

A control solution was prepared by mixing 2 ml of aqueous-ethanol with 0.5 ml of DPPH solution. Additionally, a blank solution was prepared by mixing 2ml of aqueous ethanol without any extract or DPPH. These control and blank solutions serve as reference points for comparison.

The percentage inhibition of the DPPH radicals due to the antioxidant activity of the extract was calculated using the following formula:

Percent inhibition = [(A control – A sample)/A control] x 100

Where A control represents the absorbance of the control solution (without extract) and A sample represents the absorbance of the sample solution (with extract).

By comparing the absorbance values, the extent of DPPH scavenging activity can be determined. A higher percentage inhibition indicates a stronger antioxidant activity of the extract.

2.6 MTT Assay:

The MTT assay was conducted as previously described [Nguyen et al.,2017.]. Briefly, human glioblastoma cell lines (10000 cells/well) were cultured in 96-well plates and incubated for 24 h. Following incubation, the cells were exposed to various Artocarpus heterophyllus seeds extracts and incubated for a further 24 h. Then, the cells were washed with phosphate-buffered saline (PBS), 10µl of 5 mg/ml MTT solution was added to each well, and the cells were incubated at 37°C for 2 h. Following incubation, 100µl of dimethyl sulfoxide (DMSO) was added to each well to solubilize formazan and the culture plates were incubated for 15 min at room temperature. The absorbance was recorded at 570nm using a microplate reader.

The percentage of cell viability was calculated using the formula:

Cell Viability (%) = [Absorbance of treated cells/Absorbance of control cells] x 100

Where:

- Absorbance of treated cells refers to the absorbance reading of the cells exposed to various banana flesh extracts after the MTT assay.

- Absorbance of control cells refer to the absorbance reading of the cells that were not exposed to any treatment (usually treated with a control solution or medium) after the MTT assay.

This formula gives you the percentage of viable cells relative to the control, based on the amount of formazan dye formed. The MTT assay measures cell metabolic activity, and the intensity of the formazan dye is directly proportional to the number of viable cells. The higher the absorbance, the higher the metabolic activity and viability of the cells.

3. RESULT

3.1 Molecular Docking studies:

The objective of this study was to explore the binding affinity of chemical constituents from Artocarpus heterophyllus seeds with human tubulin, employing molecular docking techniques targeting the PDB ISAO. A critical initial step involved establishing and validating a robust docking protocol.

To assess the reliability of the chosen docking methodology in accurately predicting ligand binding within the active site, the bound ligand was extracted from the X-ray crystallographic protein structure. This extracted ligand was then subjected to re-docking using Flare 7.0. Remarkably, the docking protocol effectively recapitulated the binding mode of the original ligand, yielding a placement nearly indistinguishable from the co-crystallized X-ray structure.

For the molecular docking experiments involving the receptor, DAMA Colchicine was utilized as a reference compound. Three distinct chemical constituents from Artocarpus heterophyllus seeds were subjected to evaluation to determine their inhibitory potential against human tubulin. The outcomes obtained from the simulated screening (see Table 1) unveiled that all three chemical constituents surpassed the performance of the reference compounds based on docking ratings. These compelling results suggest the superior binding capabilities of these phytochemicals, positioning them as potential efficacious inhibitors.

Among the constituents that were screened, Kaempferol D stood out as the most remarkable, displaying an exceptional docking score of -7.952 when tested against the human tubulin protein. A thorough analysis of the interaction between Kaempferol D and the Tubulin Protein revealed its binding with crucial amino acid residues. These include THR at A179, VAL at A181, MET at B259, VAL at B315, ALA at B316, ASN at B258, LYS at B352, LEU at B255, and LYS at B255, all situated within the active site of the tubulin protein.

Collectively, these findings underscore the promising binding capabilities of the chemical constituents sourced from Artocarpus heterophyllus seeds against human tubulin, thereby underscoring their potential as potent inhibitors. Docking scores of the screened phytoconstituents and their interactions within the binding site of the human tubulin protein are presented in Table 1 and Figures 4-7.

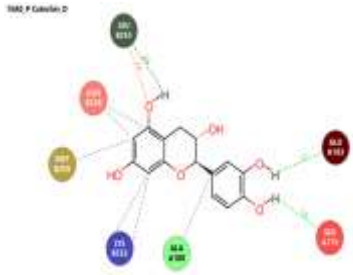


Figure ***4***: Binding model and interaction of Catechin with human tubulin protein (1SA0)

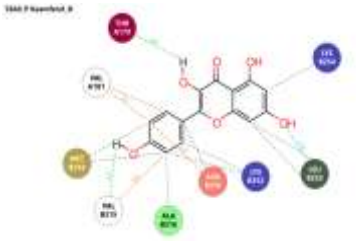


Figure ***5***: Binding model and interaction of Kaempferol with human tubulin protein (1SA0)

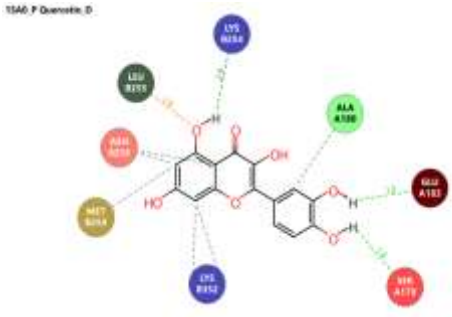


Figure ***6***: Binding model and interaction of Quercetin with human tubulin protein (1SA0)

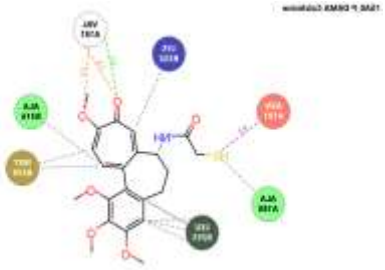
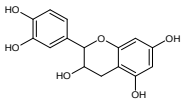
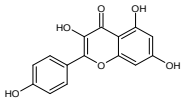
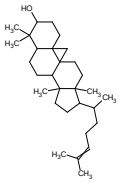
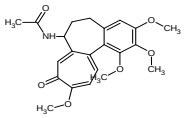


Figure *7*: Binding model and inteeractions of DAMA Colchicine with human tubulin protein (1SA0)

Table 1: In silico screening results of the phytochemicals from Artocarpus heterophyllus against human tubulin protein

Sr no.	Structure	Tittle	Protein	M.W	Atoms	Flexilibility	LF.Rank score	LF DG	LF VS score
1.		Catechin D	1SA0	290.3	21	3.6	-7.938	-7.581	-8.464

2.		Kaempferol D	1SA0	286.2	21	2.2	-8.738	-7.952	-8.812
3.		Quercetin D	1SA0	302.2	22	2.7	-8.718	-7.587	-8.571
4.		DAMA Colchine	1SA0	431.5	30	5.6	-11.451	-11.451	-12.053

3.2 Determination of Total Phenolic content

Phenolic compounds play a crucial role in exerting antioxidative effects, directly contributing to antioxidant action in various plant extracts. When evaluating the phenolic content of the seed extract of Artocarpus heterophyllus using different solvents at a concentration of 100 mg/g, it was found to be highest in the n-hexane extract (4.088±0.140 mg/g) (Table no.2), followed by the ethanolic extract (3.745±0.157 mg/g), and expressed as mg GAE/100g (fig.8).

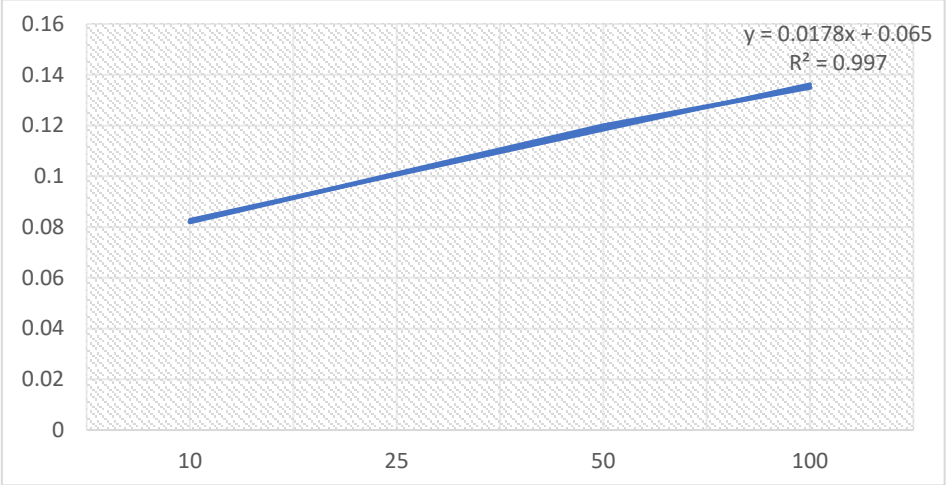


Fig. 8 Phenol Gallic acid standard curve.

3.3 Determination of Total Flavonoid content

The analysis of two different seed extracts of Artocarpus heterophyllus revealed varying levels of total flavonoid content. The n-hexane extract exhibited a higher concentration (15.126±0.066 mg/g), compared to ethanol extract (3.456±0.116 mg/g) (Table.2), all values expressed as quercetin equivalent per 100mg seed extract (Graph.2).

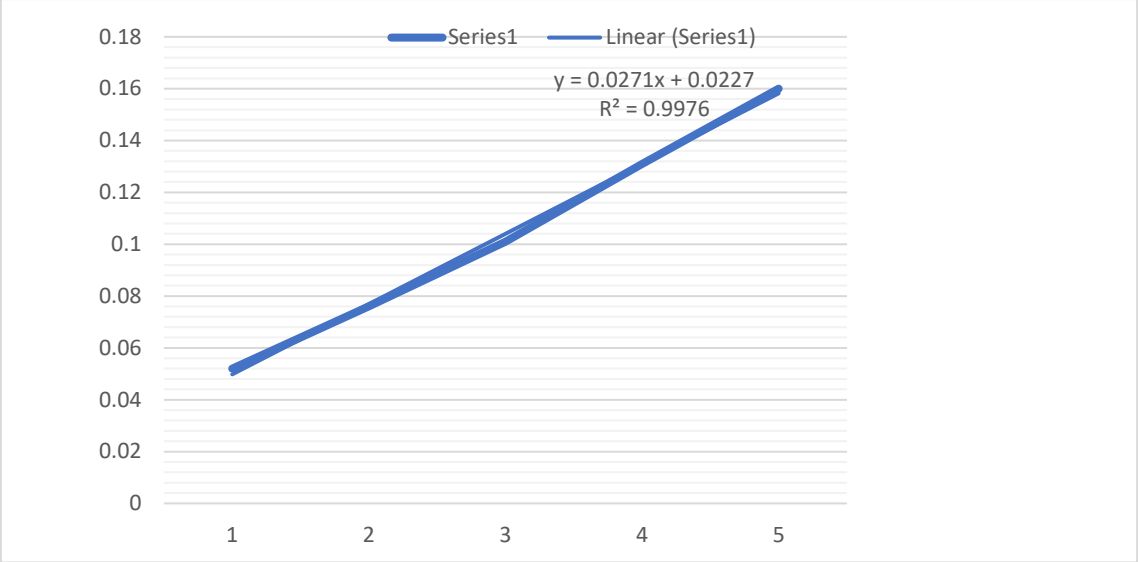


Fig.9. Flavanoid Calibration curve of quercetin standard.

Table 2 Total phenolic and flavonoid content, reducing potential of seeds extracts of Artocarpus heterophyllus

Different Fraction	Total Phenolic Content	Total flavonoid Test
Ethanol	3.745±0.157	3.456±0.116
n-Hexane	4.088±0.140	15.126±0.066

3.4 DPPH radical scavenging assay (1-diphenyl-2-picrylhydrazyl (DPPH) Radical)

The DPPH system is a reliable method for generating stable radicals, making it a popular choice for assessing the antioxidant capabilities of compounds. In this context, the DPPH assay was employed to measure the potential of antioxidants to neutralize free radicals. The effectiveness of DPPH in capturing these radicals lies in the delocalization of the unpaired electron throughout its molecular structure.

The extract derived from Artocarpus heterophyllus seeds demonstrated antioxidant activity comparable to that of the standard ascorbic acid across various tested concentrations (20, 50, and 100 µg/mL). The antioxidant potential exhibited a gradual increase with higher concentrations for all tested levels. In this evaluation of antioxidant activity using the DPPH method, ascorbic acid was employed as the reference compound. The concentrations of ascorbic acid tested ranged from 20 to 100 µg/ml. Notably, ascorbic acid displayed an inhibition percentage of 62.64297% at 20 µg/mL and reached 100.279% at 100 µg/mL [Table 3] [fig. 10]. The calculated IC50 value for ascorbic acid was 3.4514 µg/mL. Furthermore, the IC50 values for the ETOH and n-hexane extracts were determined as 80 µg/mL and 35 µg/mL, respectively. The observed results indicate that the ETOH extract exhibited a comparatively lower IC50 value than the n-HEXANE extract, suggesting a potentially stronger antioxidant effect.

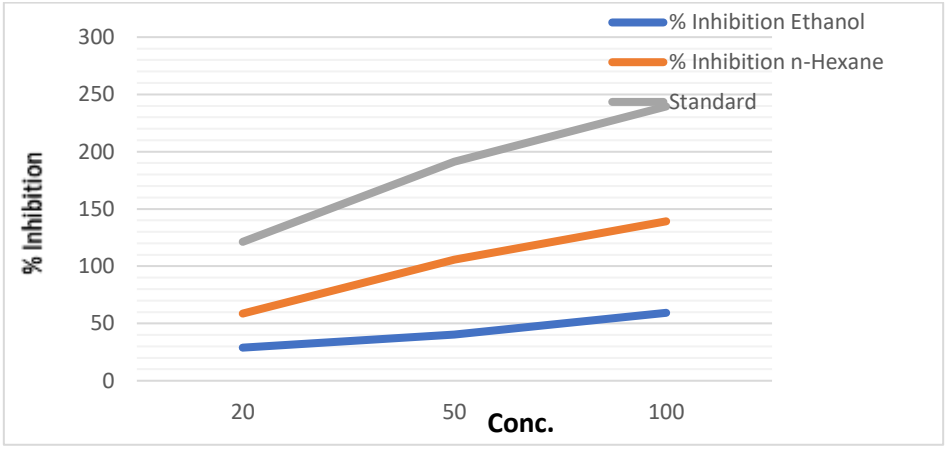


Fig. 10 Results of DPPH assay showing % inhibition with respect to ascorbic acid

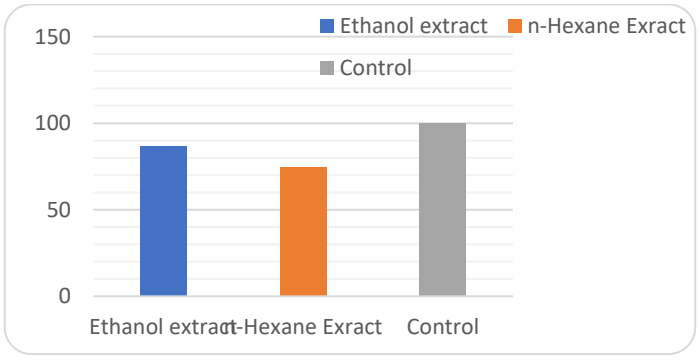
Table 3 Results of DPPH assay showing % inhibition with respect to ascorbic acid, ethanolic, and n-hexane seed extracts of Artocarpus heterophyllus

Different concentration	**Ethanol**	**n-Hexane**	**Standard**
20mg	28.90788	29.73306	62.64297
50mg	40.29827	65.46152	85.61948
100mg	59.30138	79.87402	100.279

3.5 Anticancer Activity in Human glioblastoma cell line (MTT Assay):

The U87 (MG) cells were seeded in a 96 well plate with the concentration of 10000 cells per well using DMEM media with 10% FBS and 1% Penicillin - Streptomycin. The plate was incubated at 37°C in a CO_2 incubator (with 5% CO_2 and 95% RH). The test material was dissolved in Di- Methyl sulphoxide (DMSO) before application on cells (Graph no.11) (table no.4). The test material was tested at a concentration (100 µg/well) with an untreated control for 24 hr incubation time. Further, MTT solution was prepared from the 5 mg/ml stock solution in fresh DMEM media with 10µL/well/100µL calculation and after the incubation period (24hr) was over the drug was replaced with MTT added media (figure 12). The plate was incubated at 37 °C in the CO_2 incubator for next 3 hr and then the MTT added media was replaced with DMSO (100µl/well) to dissolve the formazan crystals (purple). The plate was further incubated at room temperature for 30 min wrapped with aluminium foil. The absorbance was observed and recorded at 570 nm in the Multiskan Sky Microplate Spectrophotometer (Thermo-scientific).

The outcome obtained from the MTT assay demonstrates that the solution obtained by dissolving the ethanol and n-hexane extract sourced from the seeds of Artocarpus heterophyllus exhibited pronounced anti-proliferation effects. Notably, at a concentration of 100 µg/ml per well, the n hexane solution displayed significantly elevated anti-proliferative activity, as evidenced by a viability rate of 74.5875%. Additionally ethanol showed less antiproliferation (86.7555%). This noteworthy result highlights the extract's potential to curb cell growth in an in vitro cytotoxicity investigation when contrasted with the untreated control group.



GRAPH 11 results of MTT assay in Graphical representation of Effect of the different concentration (ethanolic extract), (n-hexanic extract) of Artocarpus heterophyllus seeds in U87(Mg cells)

Table 4 Effect of the different concentration (ethanolic extract), (n-hexanoic extract) of Artocarpus heterophyllus seeds in U87(Mg cells)

	ETOH Extract	**n- Hexane Extract**	**Control**
% Proliferation	86.7555	74.5875	100

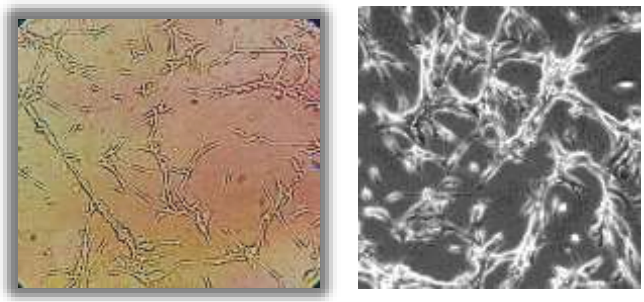


Fig. 12. U87 (MG) cell line in MTT assay

4. Discussion

The application of molecular docking in this research has provided valuable insights into the binding interactions between chemical constituents from *Artocarpus heterophyllus* seeds and human tubulin protein. Molecular docking is a powerful computational technique that plays a pivotal role in drug discovery, offering a predictive platform to understand the binding mechanisms between ligands and target proteins. The insights gained from these studies can guide the design and optimization of potential drug candidates.

The successful validation of the docking protocol through the accurate reproduction of the binding mode of the extracted ligand highlights the reliability of the approach. This validation step ensures that the chosen protocol is capable of capturing the correct binding conformation of ligands within the active site of the protein. The docking scores obtained for the reference compound DAMA Colchicine and the chemical constituents from *Artocarpus heterophyllus* seeds indicate the potential inhibitory capacity of these compounds against human tubulin.

Among the screened constituents, Kaempferol D displayed the highest docking score against human tubulin. This suggests that Kaempferol D may possess a strong affinity for the binding site of the protein, potentially indicating its role as a promising inhibitor. The detailed analysis of the interactions between Kaempferol D and the active site residues of tubulin provides insights into the molecular mechanisms underlying this interaction. These interactions involve specific hydrogen bonds, hydrophobic interactions, and potentially other non-covalent forces that stabilize the ligand within the binding site.

The molecular docking results not only offer valuable information about the binding modes but also serve as a basis for further experimental validation. The compounds identified through docking can be prioritized for synthesis and testing to verify their inhibitory potential against human tubulin in a laboratory setting. Additionally, molecular docking aids in understanding the structural features necessary for strong binding, which can guide the modification of ligand structures to enhance their binding affinity.

However, it is important to acknowledge the limitations of molecular docking studies. The accuracy of docking results heavily relies on the quality of the input structures, including protein and ligand structures. The choice of scoring functions also impacts the reliability of the results. Furthermore, molecular docking provides a static view of the binding interactions, overlooking the dynamic nature of protein-ligand interactions in real-world conditions. Thus, experimental validation through techniques like binding assays and cell-based assays is crucial to corroborate the predictions made through molecular docking.

Molecular docking studies have offered a valuable glimpse into the potential binding interactions between chemical constituents from *Artocarpus heterophyllus* seeds and human tubulin protein. The computational insights provided by docking results serve as a foundation for further experimental exploration and validation. Ultimately, these findings contribute to the broader understanding of the bioactive potential of *Artocarpus heterophyllus* seeds and their applications in drug discovery and development [Chaudhary et. al.,2022].

The significance of phenolic compounds lies in their ability to act as potent chain-breaking antioxidants and free radical terminators [Shahidi *et. al.*,1992]. These compounds possess hydroxyl groups that enable them to scavenge free radicals effectively [Hatano *et. al*., 1989]. The mechanisms of action of flavonoids, a subgroup of phenolic compounds, involve scavenging or chelating processes [Cook *et. al*., 1996] [Kessler *et. al.*, 2003]. Generally, extracts with high antioxidant activity tend to have a high phenolic content.

Among the various types of polyphenols found in plants, these compounds stand out due to their exceptional redox properties, allowing them to efficiently absorb and neutralize free radicals. They are also adept at quenching singlet and triplet oxygen or decomposing peroxides [Zheng *et al*., 2001]. As a result of these properties, phenols found in plants demonstrate excellent antioxidant, anti-mutagenic, and anti-cancer activities.

Phenolic compounds present in plant extracts, especially the n-hexane extract of *Artocarpus heterophyllus* seeds in this case, possess remarkable antioxidative capabilities. Their ability to neutralize free radicals through various processes makes them valuable contributors to the overall antioxidant potential of these extracts. Moreover, the presence of polyphenols further enhances their efficacy in combating oxidative stress and related health benefits, making them important natural compounds with promising applications in various fields. In our studies we found that the n-hexane extract exhibited higher antioxidant potential in comparison of ethanol extract of seed of *Artocarpus heterophyllus*. Our findings have similar observations in manner of antioxidant potential as reported by [Shanmugpriya *et. al*., 2011].

Flavonoids represent a vast group of more than 4000 polyphenolic compounds that naturally occur in plant-based foods, making them ubiquitous in human diets. Their presence in these diets has significant implications for human health, as flavonoids exhibit impressive pharmacological activities as radical scavengers [Yao *et. al*., 2004]. With their broad range of chemical and biological properties, including antioxidant and free radical scavenging capabilities, flavonoids are considered one of the most important natural phenolics [Kahkonen et. al., 1999.].

Studies have identified flavonoids as potent antioxidants, capable of scavenging various reactive oxygen species and inhibiting lipid peroxidation. These compounds also hold potential as therapeutic agents against a diverse array of diseases [Ross *et. al*.,2002] [Williams *et al*., 2004]. Phenols and polyphenolic compounds, including flavonoids, are commonly found in plant-derived food products and are known for their substantial antioxidant activity.

The relationship between total phenol content and antioxidant activity has been extensively explored, particularly in various food items like fruits and vegetables [Acker *et al.*,1996.].

The analysis of total flavonoid content in different *Artocarpus heterophyllus* seed extracts underscores the significance of these compounds in plant-based foods. Their abundant presence in human diets and their remarkable pharmacological properties as radical scavengers highlight their potential health benefits. Flavonoids' diverse chemical and biological activities, including their antioxidant and free radical scavenging properties, further emphasize their importance in promoting well-being. Additionally, their potential as therapeutic agents against various diseases adds to their significance in the realm of natural phenolics. In our experimental findings two extracts ethanol and n-hexane were used to quantify the total flavonoid content present in seeds of *Artocarpus heterophyllus.* We found a remarkable difference in total flavonoid content (15.126 and 3.456) in n-hexane and ethanol extract respectively. Our result ethanolic content in term of ethanol extract has similar finding as reported by [Shanmugapriya *et. al.*, 2011].

In this study focusing on *Artocarpus heterophyllus* seed extracts, significant free radical scavenging effects were observed in a concentration-dependent manner, particularly up to 100µg/ml. The IC50 values, representing the concentration required to scavenge 50% of the DPPH radicals, were found to be 50% at 80µg/ml for the ethanolic extract and 50% at 35µg/ml for the n-Hexane extract.

The versatility of DPPH radicals as a testing agent has made it widely used for evaluating the antioxidant activity of various compounds, including plant extracts and foods. In this study, the ethanolic and n-hexane extract of *Artocarpus heterophyllus* seed extracts exhibited strong antioxidative activity, with percentages of 59.30138% and 79.87402% respectively, at their respective concentrations of 100µg/ml. Notably, the n-Hexane extract displayed particularly significant antioxidant activity in the *Artocarpus heterophyllus* seed extracts.

The observed radical scavenging activity of the plant extracts is likely attributable to their phenolic nature, which enables them to participate in electron transfer and hydrogen donation processes [Porto et. al.,2000] [Soares et. al.,1997]. Overall, the results indicate a dose-dependent relationship between the concentration of the extracts and their ability to scavenge DPPH radicals, underscoring the potential health benefits of these natural compounds as antioxidants.

In our experimental findings two extracts ethanol and n-hexane were used to find antioxidative activity by DPPH radical scavenging assay (1-diphenyl-2-picrylhydrazyl (DPPH) Radical) in seeds of *Artocarpus heterophyllus.* We found a remarkable difference in antioxidative activity (79.87402 and 59.30138) 100µg in n-hexane and ethanol extract respectively. Our results in term of DPPH radical scavenging capacity of ethanol extract have similar finding as reported by [Devi *et. al*.,2021]

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay has proven to be a robust and widely used method for evaluating cell viability and cytotoxicity. In this study, the MTT assay was employed to assess the impact of a test material, derived from *Artocarpus heterophyllus* seeds, on U87 (MG) cells.

The MTT assay is based on the ability of viable cells to reduce the MTT reagent, resulting in the formation of formazan crystals [Rodríguez *et. al*.,2021.]. The purple formazan crystals produced are proportional to the number of viable cells in the culture. In our study, the reduction of MTT by cells was used as an indicator of cell viability after exposure to the test material.

The results of the MTT assay revealed significant anti-proliferative effects of the n-hexane extract from *Artocarpus heterophyllus* seeds. Notably, the n-hexane solution exhibited a pronounced decrease in cell viability, suggesting its potential cytotoxic activity. The viability rate of 74.5875% at a concentration of 100 µg/ml indicated a substantial reduction in viable cells. In contrast, the ethanol extract displayed a lesser impact on cell viability, with a viability rate of 86.7555%. These findings underscore the differential cytotoxic potential of the two extracts and emphasize the need for further investigation into their bioactive constituents.

It's important to acknowledge that the MTT assay, while valuable, provides a snapshot of cell viability under specific conditions. The results are influenced by various factors such as the characteristics of the cell line, the experimental setup, and the potential interactions between the test material and the cells. Further studies involving additional concentrations, exposure times, and complementary assays are warranted to comprehensively assess the cytotoxic effects and elucidate the underlying mechanism.

The MTT assay presented a reliable means to evaluate the cytotoxic effects of the test material derived from *Artocarpus heterophyllus* seeds on U87 (MG) cells. The assay's simplicity and sensitivity make it a cornerstone in cell viability assessments, aiding researchers in understanding the potential impacts of various agents on cellular health. Nonetheless, careful interpretation and consideration of experimental variables are essential for deriving meaningful insights from MTT assay results [Burci *et. al*.,2019] [Patel and Patel., 2011.].

The observed results of the MTT assay demonstrated that the n-hexane extract of *Artocarpus heterophyllus* seeds exerted significant anti-proliferative effects on U87 (MG) cells. The n-hexane extract exhibited a concentration-dependent reduction in cell viability, with a pronounced impact observed at a concentration of 100 µg/ml. This effect is indicative of the extract's cytotoxic potential against these glioblastoma cells. In contrast, the ethanol extract displayed a milder reduction in cell viability, suggesting a less potent cytotoxic effect.

The differential cytotoxic effects observed between the two extracts underscore the potential importance of the extraction solvent in determining the bioactive constituents present in the extracts. The n-hexane extract, which exhibited more pronounced cytotoxicity, may contain compounds with stronger anti-proliferative properties. However, further studies are necessary to elucidate the specific bioactive compounds responsible for the observed effects and to decipher their underlying mechanisms of action.

5. Conclusion:

This study conducted on the seed extracts of *Artocarpus heterophyllus* (Jackfruit) has provided valuable insights into its potential bioactive properties. The research encompassed various experimental techniques, ranging from molecular docking studies to assess binding potential, to the determination of antioxidant and cytotoxic activities through *In vitro* assays.

The molecular docking studies demonstrated that chemical constituents present in *Artocarpus heterophyllus* seeds exhibited promising binding capabilities against human tubulin protein. These findings suggest the potential of these compounds as effective inhibitors, potentially contributing to their role in drug discovery and development.

The assessment of total phenolic and flavonoid content revealed the presence of these bioactive compounds in different seed extracts. The n-hexane extract demonstrated higher phenolic and flavonoid content compared to the ethanol extract. These phenolic compounds are known for their antioxidative properties, which contribute to their potential health benefits as free radical scavengers.

The DPPH radical scavenging assay further substantiated the antioxidant potential of *Artocarpus heterophyllus* seed extracts. Both ethanol and n-hexane extracts exhibited notable radical scavenging activity, with the n-hexane extract displaying a more potent effect. These results underline the extracts' potential as natural sources of antioxidants.

In the cytotoxicity evaluation using the MTT assay, the n-hexane extract displayed significant anti-proliferative effects on U87 (MG) cells. The reduced cell viability observed suggests potential cytotoxic activity. On the other hand, the ethanol extract exhibited a lesser impact on cell viability, indicating a differential effect between the two extracts.

This comprehensive study sheds light on the potential bioactivity of *Artocarpus heterophyllus* seed extracts. The observed binding potential, antioxidant activity, and cytotoxic effects indicate the presence of valuable bioactive compounds in the seeds. These findings offer a foundation for further research, potentially leading to the development of novel pharmaceutical agents or functional food ingredients. However, further investigations are necessary to elucidate the specific bioactive compounds responsible for these effects and to uncover the underlying mechanisms of action.

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