

## ORIGINAL ARTICLE

**Pharmacognostical Evaluation and In vitro Cytotoxic Activity of Alcoholic and Aqueous Extracts of Corm of *Amorphophallus paeoniifolius* (Dennst)**

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**Abstract**

**Background:** Cancer is an abnormal growth of cells that can arise in any part of the body and causes death. It affects more than one-third in the world and is 20% higher in all fatalities. Tobacco, viral infection, chemicals, radiation etc. causes cancer. Several cancer treatments include surgery, chemotherapy, and radiotherapy.

**Objective:** In this investigation, a corm of *Amorphophallus paeoniifolius* (AP) was evaluated for pharmacognostical, preliminary phytochemical investigation and curative effect of aqueous extract of *Amorphophallus paeoniifolius* (APA) and alcoholic extract of *Amorphophallus paeoniifolius* (APE) corm for *in vitro* anti-cancer activity.

**Methodology:** In the present study, breast cancer, cancer of prostate and cancer of cervical human cell lines were used by using MTT, SRB and Trypan blue methods followed by their histopathological studies.

**Results:** The pharmacognostical studies helps in authentication and identification of plant. The results of cytotoxicity studies of aqueous extract of corm of AP revealed significant cytotoxic properties as compared to alcoholic extract and control group against breast carcinoma, prostate carcinoma, carcinoma of cervical cell lines in all the tested *in vitro* anti-cancer models with CTC<sub>50</sub> value > 500 µg/mL. The % inhibition depends on the increase in concentration of crude extracts and attains linearity till it reaches CTC<sub>50</sub> value.

**Conclusion:** From the results, we can conclude that aqueous and alcoholic extracts of corm of plant can be employed in treatment of various cancer diseases.

**Keywords:** *Amorphophallus paeoniifolius*, cytotoxicity, MTT assay, sulforhodamine B (SRB) assay, Trypan blue assay

**Introduction**

Even though allopathic medicine has advanced significantly, herbal medicines are the preliminary source for the treatment of diseases in developing countries, including India. The number of people using complementary or alternative medicine is rapidly increasing worldwide. The spectrum of medicinal plant applications has grown as our understanding of metabolic processes and plant effects on human physiology

has grown. According to a research published by the World Bank in 1997 (technical paper number 355), the importance of plant-based medicines has grown around the world. Natural basic ingredients make up about half of all medicines on the market. Because many of the active compounds in medicinal plants cannot yet be synthesized, the market needs for therapeutic herbs are increasingly valuable.<sup>1</sup>

*Amorphophallus paeoniifolius* (AP) Dennst. (Family:

Araceae) is commonly known as Suranah in Indian alternative medicine. It is a herbaceous plant with underground, lightly spherical, dark brown corm, cultivated throughout India. Traditionally the corm is used as expectorant, carminative, aphrodisiac, haemostatic, anthelmintic, liver disorders and in tumors treatments.<sup>2</sup> The corm residue possesses analgesic enzyme inhibitory in tuber crops<sup>3</sup> and anti-obesity activity. The corm of the plant contains steroids, flavonoids and tannins. The corm of AP is used in the diagnosis of cancer by traditional Indian practitioners (Figure 1).<sup>4</sup>



**Figure 1:** Photo of corm of *Amorphophallus paeoniifolius*

Irrespective of drug development, there are scanty reports on clinical trials related to human cancers. Particularly, cancer reappearance is not because of complete surgical eradication and acquaintance to anticancer drugs. Cancers are treated with medicines that mitigate growth of tumor cell and are against DNA synthesis as well as important signaling molecules that lead to tumor cell growth. As the current treatment methods like chemo and radio-therapies have serious side effects, the non-toxic, safe, and low cost anticancer drugs are urgently needed. Many phytochemicals are non-toxic, easily available and compatible to our physiological system, thus prudent, but can also be a better approach in prevention of cancer and treatment.<sup>5-9</sup>

## Materials and Method

### Chemicals

Sigma Company, Louis St, USA, provided 3-(4,5-dimethylthiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), and Trypsin. Hi Media Laboratories Ltd., Bombay, provided Ethylene Diamine Tetra Acetate, Glucose, and antibiotics, whereas Merck E Ltd., Bombay, provided dimethyl sulfoxide (DMSO) and Propanol.

### Cell lines and Culture medium

MCF-7 (Breast carcinoma) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were grown in DMEM medium containing 10% deactivated FBS, penicillin (100 IU/mL), streptomycin (100 g/mL), and amphotericin B (5 g/mL) in a humidified condition (5% CO<sub>2</sub>). TPVG solution was further mixed to the cells (0.2 percent trypsin, 0.02 percent EDTA, 0.05 percent glucose in PBS). All studies were carried out in 96 microtitre plates, with the cultures stock being produced in 25 cm<sup>2</sup> culture flasks (India Tarsons Pvt. Ltd., Calcutta).

### Preparation of test solutions

The test drug was separately weighed and mixed in distilled Dimethyl sulfoxide. The final volume was adjusted with DMEM and 2% inactivated FBS for cytotoxicity tests, resulting 1 mg/mL concentration of stock solution that was sterilized by filtering. For cytotoxic investigations, serial two-fold dilutions were made.

### MTT assay

Using DMEM media containing 10% FBS, the cell culture monolayer was trypsinized and count was set as  $1.0 \times 10^5$  cells/mL and from that 0.1 mL of the cell suspension was put in to 96 well plate reader. After 24 hours, the supernatant was flicked off, the monolayer was washed once with medium, and 100 microliter of various test drug concentrations were added to the partial monolayer microtitre plates. The incubation of the plates was carried out at 37°C in a 5% CO<sub>2</sub> atmosphere for three days with microscopic examination and observations were made every 24 hours. The medication solutions in the wells were removed after 72 hours, and 50 microlitres of MTT in PBS were added to each well. The plates were lightly shook before being incubated at 37°C for 3 hours in a 5% CO<sub>2</sub> environment. The supernatant was withdrawn, 100 litres of propanol was added, and the plates were gently agitated to dissolve the formazan that had formed. A microplate reader was employed to detect absorbance at a wavelength of 540 nm.<sup>10</sup>

### Procurement of culture for Human cell lines study

National Centre for Cell Sciences (NCCS), Pune, India, provided the PC-3 (Prostrate cancer) cell line. Cell stock was grown until confluent in DMEM supplemented with 10% inactivated FBS, penicillin, streptomycin, and amphotericin B in a humidified environment of 5% CO<sub>2</sub>.

TPVG solution trypsin was used to dissociate the cells (0.2 percent, EDTA 0.02 percent, glucose 0.05 percent in PBS). Cultures of stock were grown in 25 cm with two culture flasks, whereas tests were carried out in 96 microtitre plates.

#### **Determinations of cell count by sulforhodamine B (SRB) assay**

Using media containing 10% FBS, the monolayer cell culture was trypsinized and was adjusted to  $1.0 \times 10^5$  cells/microliter. 0.1mL of diluted cell suspension (about 10,000 cells) was put in to 96-well microtitre plate. After 24 hours, the supernatant was removed and washed once with medium, and 100 L of various residues were given to the cells in microtitre plates. The plates were then incubated for three days at 37°C in a 5% CO<sub>2</sub> environment, with microscopic examination and observations recorded every 24 hours. After 72 hours, 25 microliters of 50% trichloroacetic acid was gently poured into the wells to build a layer over the extract, resulting in a 10% concentration. After that, the plates were incubated for 1 hour at 40 degrees Celsius. To remove remnants of medium, the plates were taken and washed for five times with water. The extract and serum were then air dried. For 30 minutes, they were stained with SRB. After that, the unbound dye was quickly removed by washing four times with 1% acetic acid. The plates were then allowed to air dry. To dissolve the dye, 100 mL of Trisbase (10 mM) was introduced to the wells. For five minutes, they were constantly rattled. At a wavelength of 545 nm, the absorbance was calculated using a microplate reader.<sup>11</sup>

#### **Human cell lines and Culture media**

The HeLa (Carcinoma Cervical) cell line was procured from the National Center for Cell Science in Pune, India. Stock cells were grown until stable in DMEM supplemented with 10% deactivated FBS, penicillin, streptomycin, and amphotericin B in a humidified environment. TPVG solution was used to disintegrate the cells (0.2 percent trypsin, 0.02 percent EDTA, 0.05 percent glucose in PBS). Cultures of stock were grown in 25 cm in two culture flasks, and tests was detected on 96 microtitre plates (India Tarsons Pvt. Ltd, Culcutta, India).

#### **Cell counting with exclusion of trypan blue**

Using DMEM media containing 10% FBS, the culture cell was trypsinized and was adjusted to  $1.0 \times 10^5$  cells/mL. 1 mL of diluted cell suspension (about 100,000

cells) was added to each 40 mm petri dish. After 24 hours, when a partial monolayer had developed, the supernatant was removed, the monolayer was washed once with medium, and 1 mL of various test drug doses were applied to the partial monolayer in culture dishes. The dishes were then incubated for three days at 37°C in a 5% CO<sub>2</sub> environment, with microscopic examination and observations performed every 24 hours. The medication solutions in the wells were withdrawn after 72 hours, and trypsinized and further washed in PBS and centrifuged to remove the cell pellet. They were then resuspended in 1 mL of new medium and dye exclusion tests were performed, in which an equivalent number of drug-treated cells and trypan blue (0.4 percent) were combined and left for 1 minute. It was then placed into a haemocytometer, and the number of survivors and non-survivors was counted in under two minutes.<sup>12</sup> The percentage of cytotoxicity and growth inhibition were calculated by the following formula:

#### **Determination of % cytotoxicity:**

$$\% \text{ Cytotoxicity} = \frac{\text{No. of dead cells} \times 100}{\text{No. of viable cells} \times \text{dead cells}}$$

#### **Determination of % growth inhibition:**

$$\% \text{ Growth inhibition} = \frac{100 - \text{O.D of individual test group}}{\text{O.D of control group} \times 100}$$

#### **Procurement and authentication of plant**

The dried corm of *Amorphophallus paeoniifolius* was collected from Dharwad district in June 2020. The collected material was authenticated by Dr. S S Hebbar, Botanist, Govt. P U College, Dharwad, Karnataka (SETCPD/REF/40/20).

#### **Preliminary phytochemical investigation of corm extracts of *Amorphophallus paeoniifolius* (Dennst).**

- \* Pharmacognostic evaluation of *Amorphophallus paeoniifolius* corm
- \* Extraction of *Amorphophallus paeoniifolius*
- \* Qualitative chemical analysis
- \* Acute toxicity studies

#### **Pharmacognostic and morphological evaluation of corm *Amorphophallus paeoniifolius***

##### **a) Extractive methods:**

- Extractive of alcohol soluble
- Extractive of water soluble

**B) Determination of moisture content****C) Ash values**

- Total ash content
- Ash containing acid insoluble
- Ash containing water soluble
- Ash containing sulphates

**Proximate values<sup>13-15</sup>****i) Extractive values**

The determination of extractive values assists to determine the amount of soluble constituents in a given amount of medicinal plant material, when extracted with solvent. The extraction of any crude drugs with a particular solvent gives a residue containing different chemical constituents. The mixture of these plant constituents in that peculiar solvent depends on drug and solvent employed. The use of particular solvent can also be employed for preliminary view of quality of an individual drug sample.

**ii) Extractive values of alcohol soluble**

In a closed flask, 5 gm of shade dried AP powder was macerated with 100 mL of 95% ethanol and allowed to stand for 18 hours, shaking frequently during the first six hours. After that, it was quickly filtered to prevent the loss of ethanol. In a tared flat of shallow dish, 25 mL of filtrate was evaporated to dryness, the moisture content was removed at 105°C, and the weight was examined. With respect to the shade-dried plant powder, the percent ethanol soluble extractive was computed.

**iii) Extractive values of water soluble**

Five grams of shade dried corm powder of AP was extracted with aqueous solvent (100 mL) by maceration method in a closed flask for six hours and then allowed to sit for 18 hours before being filtered. In a tared flat bottom shallow dish dried at 105°C, 25 mL of filtrate was evaporated to dryness and the weight was verified. The extractive values were computed as a percentage of the shade-dried corm.

**b) Content of moisture**

In a tared glass bottle, a weighed quantity of powder of AP corm was added, and the weight was taken first. The weight of the crude powder was estimated after it was heated to 105°C in a hot oven. This procedure was repeated until a steady weight was achieved. The sample's moisture content was calculated as a percentage of the shade dried material.<sup>16</sup>

**c) Content of Ash**

## ➤ Ash content total

In a tared silica crucible, 2 gm of AP shade dried powdered corm was added and burned at a temperature not exceeding 450°C until free of carbon, cooled, and weighed. The overall ash % was obtained using shade dried corm as a reference.

## ➤ Acid insoluble ash

With 25 mL of diluted HCl, the whole ash was heated for five minutes. The ash-free filter paper was used to capture the insoluble debris, which was then cleaned with hot water, burned, cooled, and weighed. With respect to shade-dried corm, the proportion of acid insoluble ash was estimated.

## ➤ Water soluble ash

Entire ash obtained was boiled for 5 min with 25 mL distilled water, cooled and the insoluble matter on ash-less filter paper was collected, washed with hot water and burned for 156 min at a temperature not exceeding 450°C. The weight of the insoluble ash was subtracted. The percentage of ash soluble in water was determined with respect to shade-dried corm.

## ➤ Sulphated ash

The silica crucible was heated for 10 minutes until it turned red, then cooled and weighed. One gm corm powder was dried and placed in a silica crucible, soaked with sulphuric acid, softly burned, then moistened with sulfuric acid again and burned at 800°C. Cooled and weighed, then burnt for another 15 minutes and weighed again. With respect to air dried corm, the proportion of sulphated ash was calculated.

**Extraction of *Amorphophallus paeoniifolius***

Two kilograms of AP of corm was taken and shade dried and was exhaustively extracted with ethanol using Soxhlet apparatus. Another 1 kg of corm of this plant was kept for maceration using distilled water. The extracts were concentrated using rotary flash evaporator. The obtained residue was dehydrated in a desiccator over calcium chloride. Some parts of the extract were reserved for preliminary phytochemical investigation, acute toxicity studies, and pharmacological activity.

**Qualitative Chemical Analysis<sup>17</sup>**

The following tests were done to confirm the presence of various phytoconstituents.

### Detection of carbohydrate

- a. **Molisch's test:** To 2-3 mL of aqueous test solution, add few drops of Molisch's reagent, ( $\alpha$ -Naphthol in alcohol) shake, and add few drops of conc. Sulphuric acid from the side of test tube without shaking. Violet to purple colored ring at the junction of two liquid indicates presence of carbohydrates.
- b. **Benedict's test:** Mix equal volumes of Benedict's reagent and test solution in a test tube. Heat on a boiling water bath for 5 min. Solution appears brick red in colour.
- c. **Fehling's test:** Mix each 1 mL of Fehling's A and B solutions, and add equal volume of test solution. Heat it on a boiling water bath for 5 min. Brick red precipitate indicates the presence of reducing sugars.

### Detection of glycosides

- a. **Borntrager's test:** Boil the extract with 2 mL of dilute sulphuric acid in boiling water bath for 5 minutes, filter while hot. Cool the filtrate and shake with equal volume of chloroform or dichloromethane and separate the organic layer, and add equal volume of ammonia solution. Ammonical layer shows pink or red colour, due to the presence of anthraquinone moiety.
- b. **Modified Borntrager's test:** Boil the extract with 2 mL of 5%  $\text{FeCl}_3$  solution (freshly prepared) and 2 mL of dilute hydrochloric acid in boiling water bath for five minutes, filter while hot. Cool the filtrate and shake with equal volume of benzene or any organic solvent and separate the organic layer. Add equal volume of ammonia solution. Lower ammonical layer shows rose pink to cherry red colour. This indicates presence of C-glycosides. As some plants contain anthracene aglycone in reduced form, if ferric chloride is oxidised to anthraquinones, it shows response to test.

### Detection of alkaloids

**Preparation of test solution:** Small quantity of extract was treated with dilute hydrochloric acid and filtered. The filtrate was used for various tests to detect the presence of alkaloids.

- a. **Dragendorff's test (Potassium-Bismuth iodide solution):** 2-3 mL filtrate with few drops Dragendorff's reagent. Reddish brown coloured precipitate.

- b. **Mayer's test (Potassium-Mercuric iodide solution):** 2-3 mL filtrate with few drops Mayer's reagent. Gives cream colour precipitate.
- c. **Hager's test (Saturated solution of picric acid):** 2-3 mL filtrate with few drops Hager's reagent. Gives yellow precipitate.
- a. **Wagner's test (Iodine-Potassium iodide solution):** 2-3 mL filtrate with few drops Wagner's reagent. Gives reddish brown coloured precipitate.

### Detection of steroids

**Preparation of test solution:** The different extracts were dissolved in chloroform and filtered. The filtrates were concentrated and used for various tests to detect the presence of steroids.

- a. **Salkowski's reaction:** 2 mL of test solution and 1 mL of conc. Sulphuric acid from side of the test tube. Red colour at lower layer indicates presence of steroids.

### Detection of flavonoids

- a. **Shinoda test:** To the test solution, add few magnesium turnings and add conc. Hydrochloric acid drop wise. Pink scarlet, crimson red appears after few minutes indicating presence of flavonoids.
- b. **Alkaline reagent test:** To the test solution, add few drops of sodium hydroxide solution. Intense yellow colour is formed, which turns to colorless on addition of few drops of dilute acid indicating presence of flavonoids.

### Detection of proteins

- a. **Biuret test:** To 3 mL T.S, add 4% NaOH and few drops of 1%  $\text{CuSO}_4$  solution. Violet or pink colour appears.
- b. **Xanthoprotein test:** Mix 3 mL T.S with 1 mL conc.  $\text{H}_2\text{SO}_4$ , white precipitate is formed. On boiling, precipitate turns to yellow. On addition of  $\text{NH}_4\text{OH}$ , precipitate turns orange.
- c. **Test for protein containing sulphur:** Mix 5 mL T.S with 2 mL of 40% NaOH and two drops of 10% lead acetate solution. On boiling, mixture turns black or brownish due to  $\text{PbS}$  formation.

### Detection of tannins and phenolic compounds

**Preparation of test solution:** Small quantity of extract was treated with distilled water and filtered. The filtrates were concentrated and used for various tests to detect the presence of tannins/ phenolic compounds.

**a. Ferric chloride test:** Treat the test solution with one drop of ferric chloride solution. Hydrolysable tannins are blue colored precipitates, whereas with condensed tannins are green colored precipitates. These precipitates indicate the presence of tannins and phenolic compounds.

**b.** To 2-3 mL of aqueous or alcoholic test solution, add few drops of following reagents:

- Lead acetate solution gives white precipitate
- Bromine water or dilute potassium permanganate solution gives decoloration
- Dilute nitric acid gives yellow colour

#### Detection of amino acids

**a. Ninhydrin test:** Heat 3 mL of test solution with 3 drops of 5% ninhydrin solution in boiling water bath for 10 min. Purple or bluish colour appears.

## Results

The morphological features of corm of plant were determined followed by pharmacognostical evaluation of plant which revealed moisture content - 6.0%, water soluble extractives -10%, alcohol soluble extractives - 20.0%, total ash value - 4.23%, acid-insoluble ash - 4.68%, water soluble ash - 5.46%, and sulphated ash - 7.5% W/W respectively. (Table 1 and 2). The percentage yield of ethanolic extract were found to be 12.8% and aqueous extract 6.6% respectively (Table 3). Microscopic evaluation (transverse section of corm) of *Amorphophallus paeoniifolius* revealed the presence of periderm and cortex, vascular bundles and parenchyma cells. Powder analysis of corm revealed the presence of starch grains, lipid bodies, raphide needles and crystals of calcium oxalate were observed under polarized light microscope.

**Table 1:** Morphological features of corm of *Amorphophallus paeoniifolius*

S. No.	Features	Observation
1	Color	Dark brown
2	Odor	Faint
3	Taste	Acrid, Astringent
4	Shape	Hemispherical
5	Size	30cm in diameter, 1-2 feet in length 5-8 cm in length

**Table 2:** Physical evaluation of *Amorphophallus paeoniifolius*

Parameters	Determined Value in %W/W
Moisture content	6.00%
Water soluble extractive	10.00%
Alcohol soluble extractive	20.00%
Total ash	4.23%
Acid insoluble ash	4.68%
Water soluble ash	5.46%
Sulphated ash	7.5%

Preliminary phytochemical analysis of alcoholic and aqueous corm extract revealed the presence of alkaloids, flavonoids, tannins, steroids, carbohydrates, proteins and amino acids as the major phytoconstituents (Table 4).

**Table 3:** Colour and yield of various extracts of corm of *Amorphophallus paeoniifolius*

S. No.	Crude Extracts	Colour and Consistency	% Yield (gm)
1	Ethanolic extract	Reddish (Semisolid)	12.80%
2	Aqueous extract	Reddish brown (Semisolid)	6.60%

**Table 4:** Qualitative chemical examination of various extracts of corm of AP

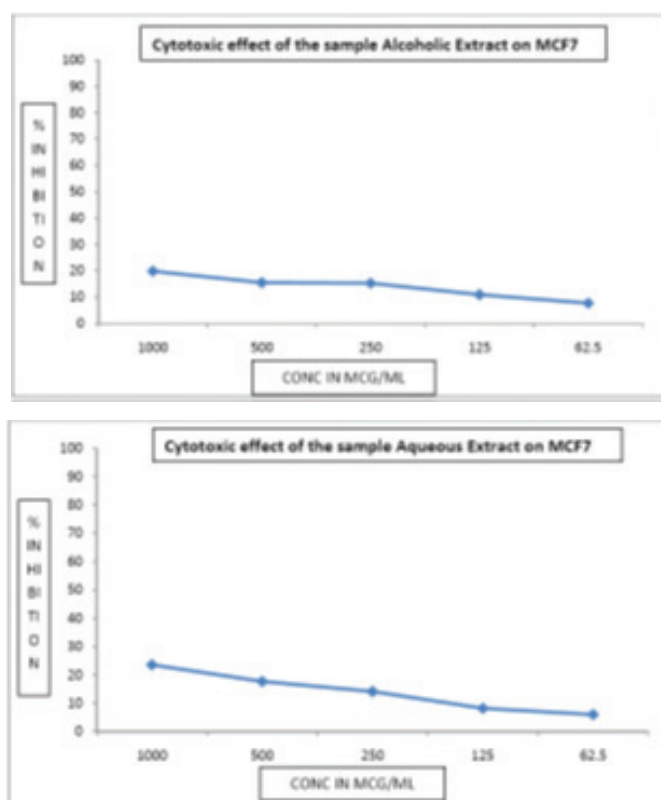
S. NO	Test For Phytoconstituents	Ethanolic extract	Aqueous extract Extract
01	Alkaloids	+	--
02	Glycosides	--	—
03	Steroids	+	—
04	Carbohydrates	+	+
05	Flavonoids	+	+
06	Tannins	+	+
07	Saponins	—	+
08	Terpenoids	—	—
09	Proteins & Aminoacids	+	+

‘+’ = Positive, ‘-’ = Absent

In MTT assay (MCF Breast carcinoma cell line), after 24 h and 72 h incubation of alcoholic and aqueous extracts at the concentration range of 50, 100, 200 and 500 µgm/mL, increased cell viability was observed. Aqueous extract of plant exhibited 23.56% cytotoxic activity, whereas alcoholic extract revealed 19.85% cytotoxicity with CTC<sub>50</sub> value > 500 µgm/mL (Table 5, Figure 2).

**Table 5:** Cytotoxic properties of test drugs against MCF-7 cell line

S. No	Name of Test sample	Test Conc. ( $\mu\text{g/mL}$ )	% Cyto-toxicity	CTC <sub>50</sub> ( $\mu\text{g/mL}$ )
1	Alcoholic extract of <i>Amorphophallus paeoniifolius</i>	1000	19.85 $\pm$ 0.7	>500
		500	15.48 $\pm$ 0.7	
		250	15.29 $\pm$ 0.9	
		125	10.92 $\pm$ 0.7	
		62.5	7.70 $\pm$ 0.5	
2	Aqueous extract of <i>Amorphophallus paeoniifolius</i>	1000	23.56 $\pm$ 1.0	>500
		500	17.66 $\pm$ 0.7	
		250	14.10 $\pm$ 0.7	
		125	8.20 $\pm$ 0.7	
		62.5	5.98 $\pm$ 0.6	

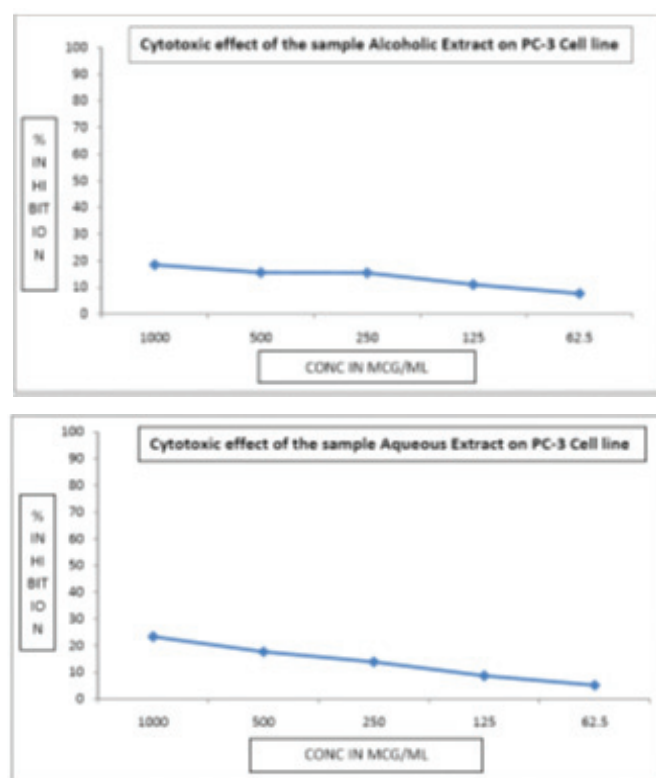
**Figure 2:** Graphical representation of alcoholic and aqueous extract of *Amorphophallus paeoniifolius* in Breast carcinoma (MCF-7 cell line)

In SRB assay (PC-3-Prostate carcinoma cell line), after 24 h and 72 h incubation of alcoholic and aqueous extracts at the concentration range of 50, 100, 200 and 500  $\mu\text{g/mL}$ , cytotoxic properties were evaluated. Aqueous extract of plant exhibited 23.30% cytotoxic activity, whereas alcoholic extract revealed 18.48%

cytotoxicity with CTC<sub>50</sub> value > 500  $\mu\text{g/mL}$  (Table 6, Figure 3).

**Table 6:** Cytotoxic properties of test drugs against PC-3 cell line

S. No	Name of Test sample	Test Conc. ( $\mu\text{g/mL}$ )	% Cyto-toxicity	CTC <sub>50</sub> ( $\mu\text{g/mL}$ )
1	Alcoholic extract of <i>Amorphophallus paeoniifolius</i>	1000	18.48 $\pm$ 0.6	>500
		500	15.60 $\pm$ 0.7	
		250	15.45 $\pm$ 0.9	
		125	11.08 $\pm$ 0.7	
		62.5	7.70 $\pm$ 0.5	
2	Aqueous extract of <i>Amorphophallus paeoniifolius</i>	1000	23.30 $\pm$ 1.0	>500
		500	17.69 $\pm$ 0.7	
		250	13.89 $\pm$ 0.6	
		125	8.65 $\pm$ 0.7	
		62.5	5.16 $\pm$ 0.6	

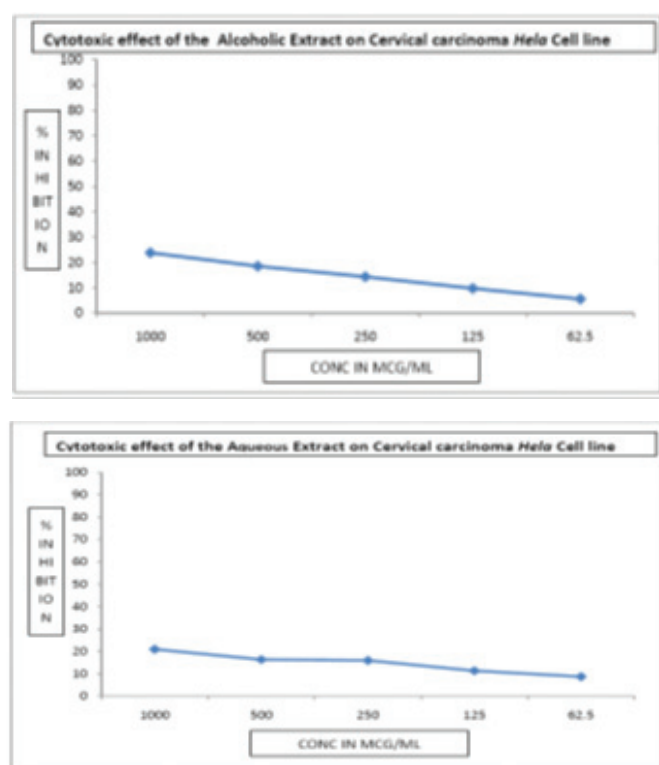
**Figure 3:** Graphical representation of alcoholic and aqueous extract of *Amorphophallus paeoniifolius* in cancer (PC-3 cell line)

In trypan blue test (Hela-1471-prostate carcinoma cell line), after 24 h and 48 h incubation of alcoholic and aqueous extracts at the concentration range of 50,

100, 200 and 500  $\mu\text{g}/\text{mL}$ , exhibited cytotoxic effects. Aqueous extract of plant exhibited 23.81% cytotoxic activity, whereas alcoholic extract revealed 20.97% cytotoxicity with  $\text{CTC}_{50}$  value  $> 500$   $\mu\text{g}/\text{mL}$ . In all the above *in vitro* tested anticancer models, aqueous residues of the corm showed better cytotoxic effects as compared to alcoholic extract (Table 7, Figure 4).

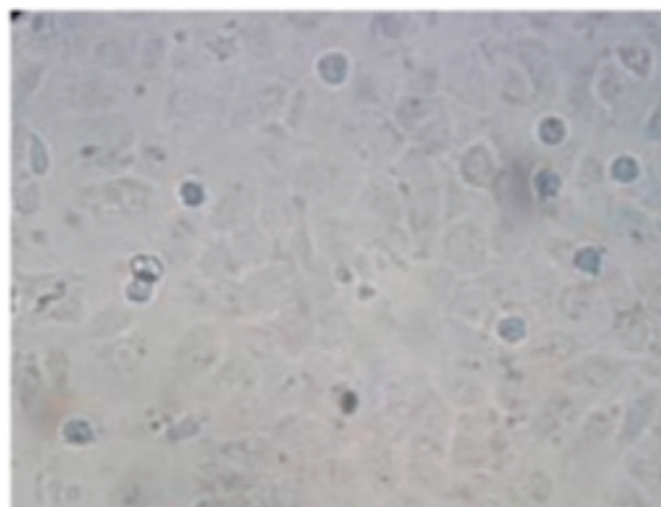
**Table 7:** Cytotoxic properties of test drugs against Hela cell line

S. No	Name of Test sample	Test Conc. ( $\mu\text{g}/\text{mL}$ )	% Cyto-toxicity	$\text{CTC}_{50}$ ( $\mu\text{g}/\text{mL}$ )
1	Alcoholic extract of <i>Amorphophallus paeoniifolius</i>	1000	20.97 $\pm$ 0.7	$>500$
		500	16.39 $\pm$ 0.7	
		250	15.97 $\pm$ 0.8	
		125	11.39 $\pm$ 0.6	
		62.5	8.71 $\pm$ 0.4	
2	Aqueous extract of <i>Amorphophallus paeoniifolius</i>	1000	23.81 $\pm$ 1.0	$>500$
		500	18.51 $\pm$ 0.7	
		250	14.35 $\pm$ 0.7	
		125	9.77 $\pm$ 0.6	
		62.5	5.60 $\pm$ 0.6	

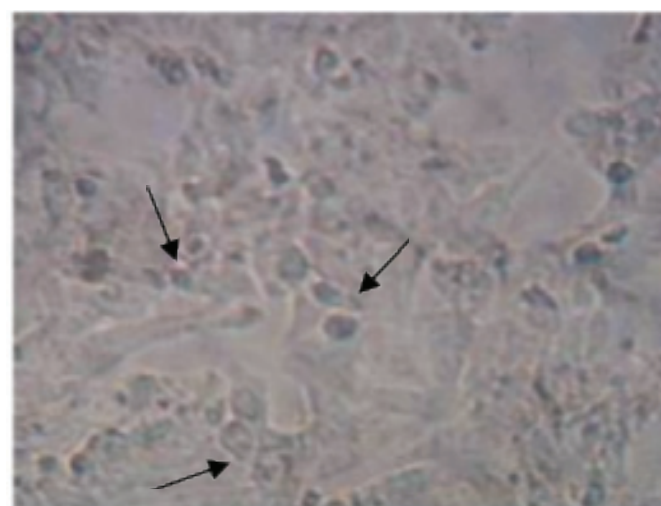


**Figure 4:** Graphical representation of alcoholic and aqueous extract of *Amorphophallus paeoniifolius* cervical cancer (HELA cell line)

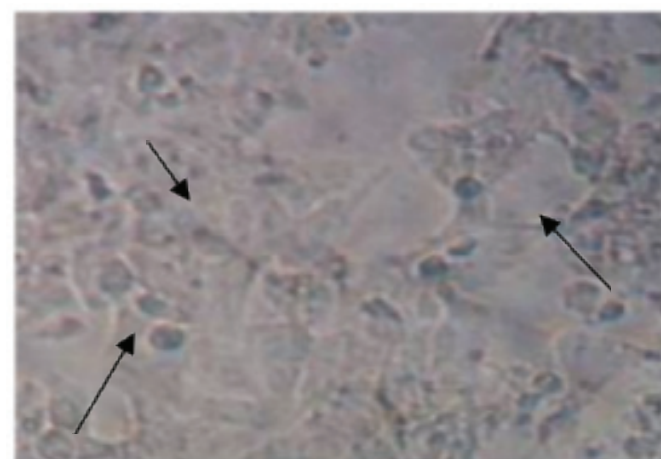
The % inhibition depends on the increase in concentration of crude extracts and attains linearity till it reaches  $\text{CTC}_{50}$  value respectively. The results were further supported by histopathological studies (Figure 5).



**(a) Histopathology of Normal group**



**(b) Histopathology of alcoholic extract of plant showing cell aggregation**



**(c) Histopathology of aqueous extract of plant showing cell round and death**

**Figure 5:** Histopathological study results

## Discussion

Cancer is the world's third greatest cause of mortality, trailing only behind cardiovascular and infectious

disorders. It is a catch-all over word for a collection of over 100 diseases that can affect any region of the body. Although there are many therapeutic strategies including chemotherapy to treat cancer, high systemic poison and capacity to drug limit and there successful outcome in most cases. Several new methods are being developed to prevent and treat cancer. One such method could be a synergistic effective of phytochemical and chemotherapeutic agents, which when mixed would increase efficacy while reducing harmful effects to normal tissues.<sup>18</sup> Pharmacognostical evaluation such as moisture content, ash values, extractive values and microscopic characters of corm of plant helps in proper identification, authentication and detection of adulteration or originality of plant or its parts.<sup>19</sup> The results of cytotoxicity studies of aqueous AP corm extract revealed significant cytotoxic properties as compared to alcoholic extract and control group against breast carcinoma cell line, prostate carcinoma cell line and cell lines of cervical carcinoma in MTT assay, SRB assay and trypan blue tested in *in vitro* anti-cancer models.

In MTT assay, aqueous extract of plant exhibited 23.56% cytotoxic activity, whereas alcoholic extract revealed 19.85% cytotoxicity with CTC<sub>50</sub> value > 500 µg/mL. In SRB method, aqueous extract of plant exhibited 23.30% cytotoxic activity, whereas alcoholic extract revealed 18.48% cytotoxicity with CTC<sub>50</sub> value > 500 µg/mL. In trypan blue test, aqueous extract of plant exhibited 23.81% cytotoxic activity, whereas alcoholic extract revealed 20.97% cytotoxicity with CTC<sub>50</sub> value > 500 µg/mL.

In all the above *in vitro* tested anticancer models, aqueous residues of the corm showed better cytotoxic effects as compared to alcoholic extract. The % inhibition depends on the increase in concentration of crude extracts and attains linearity till it reaches CTC<sub>50</sub> value respectively. The cytotoxic effects were further assisted by histopathological studies. The anti-cancer property of aqueous and alcohol extracts of AP corm could be attributed to the presence of flavonoids and tannins having mono to poly phenolic groups. The flavonoids/polyphenols have been reported for the cytotoxic activity attributed to phenolic group.<sup>20,21</sup> The probable inhibition of cancer cell may also be a contribution of other potent active principles present in plant extracts.<sup>22</sup> Further histopathological studies revealed certain cytotoxic changes such as cell aggregation, cell rounding and cell death. The overall results indicate basic knowledge for the use of crude extracts of plant as potential anti-cancer agent.

## Conclusion

Preliminary phytochemical analysis of alcoholic and aqueous corm extracts of AP revealed the presence of alkaloids, flavonoids, tannins, steroids, carbohydrates, amino acids and proteins as major phytoconstituents. From the results, we can conclude that aqueous extractives revealed potential cytotoxic activity as compared to alcoholic extract. Hence corm extracts of *Amorphophallus paeoniifolius* can be employed as potential anti-cancer agents for preclinical and clinical studies.

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## Conflict of interest

Nil.

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