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# Cytotoxicity and antimicrobial activity of *Colocasia esculenta*

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**ABSTRACT**

*Objectives: Colocasia esculenta belongs to family Araceae and is popularly known as "Taro". This plant has been cultivated widely from ancient times in the tropical and subtropical latitudinal band around the world. The herb has been known since ancient times for its curative properties and has been utilized for treatment of various ailments such as asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders, and skin disorders. Methods: However knowledge about cytotoxicity is lacking hence, cytotoxic study of Colocasia esculenta leaves and tuber was carried out. Particular attention has been given to antimicrobial and antioxidant study of tuber and leaves as well as cytotoxic effect in order to evaluate the potential use of this plant in pharmaceuticals. As bone cancer is emerging as aggressive form of cancer, anticancer study was done on osteocarcoma cell line (MG63) of human. Results: A wide range of phytochemical compounds including flavonoids, β-sitosterol, and steroids have been identified by phytochemical and analytical study. Extracts from this plant have been found to possess various pharmacological activities.*

**Key words**: Araceae, phytochemical compounds, osteocarcoma, antioxidant study, bone cancer.

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## INTRODUCTION

Herbal plants have played a significant role in maintaining human health and improving quality of human life since long and have served humans well as valuable components of medicines, seasoning, beverages, cosmetics, and dyes. The popularity of herbal medicine in recent times is based on the premise that plants contain natural substances that can promote health and alleviate illness. Therefore, the focus on plant research has increased all over the world and a large body of evidence show immense potential of medicinal plants used in various traditional system [1]. Herbal drugs or medicinal plants, and their extracts and isolated compounds have demonstrated a wide spectrum of biological activities [2]. Taro (*Colocasia esculenta* L.), a root crop belonging to the Araceae family, has been cultivated for many centuries. Originating in Asia, taro is now primarily found in tropical and subtropical regions [3, 4]. The historic use and importance of taro can explain the reason of such significant implications in human health. Taro tubers are rich in starch and the corms contain the anthocyanins, cyanidin 3-glucoside, pelargonidin 3-glucoside, and cyaniding 3-rhamnoside [5]. In common with flavonoids, the related anthocyanins are reputed to improve blood circulation by decreasing capillary fragility [6](Wagner, 1985), to improve eyesight, to act as potent antioxidants, to act as anti-inflammatory agents, and to inhibit human cancer cell growth [7,8]. The corms of taro also contain calcium oxalate, an irritant, which prevents them being eaten raw or incompletely cooked [9]. Tubers are known to supply easily digestible starch, substantial amount of protein, thiamine, riboflavin, niacin, as well as significant amounts of dietary fiber. Leaves of taro are eaten as vegetable by human, possess β carotene, iron, protein, vitamins and folic acid which protects against anemia. The major nutrient in taro corms is dietary energy.

The most abundant minerals in *Colocasia esculenta* are is potassium, phosphorus, magnesium, and calcium. The young leaves are rich in Vitamin C, and the roots are rich in starch. This plant is reported to have antimicrobial and antioxidant activity and anticancer activity [10].

In Present scenario, cancer is one of the dreadful diseases and its occurrence is increasing. In medical science the methods available to treat a cancer patient mainly includes surgery, chemotherapy and radiotherapy etc. As these known methods are very costly and have side effects with limitations of their use, there is need for effective and acceptable cancer therapeutics agents that would be non-toxic, highly efficacious against multiple cancers, palatable, cost effective. Medical plants have created great interest among researchers as they have been proved to possess anticancer activity [11] and have no side effects.

Poi, widely consumed among the people of Hawaii, is actually prepared by mashing boiled taro tubers. It has a paste like texture and delicate flavour. Taro tubers are emerging now as functional foods as they offer high nutritive values and health benefits. The Institute of Medicine's Food and Nutrition Board defined functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains." Clearly, all foods are functional, as they provide taste, aroma, or nutritive value and now days, there has been an explosion of consumer interest in functional foods [12].

It is a well-known fact that traditional systems of medicines have always played important role in meeting the global healthcare needs. They are continuing to do so at present and shall play major role in future as well. To explore the medicinal importance of taro tuber and leaf this study was undertaken. In this study antimicrobial and antioxidant effect of taro tuber and leaf was checked as well as cytotoxicity against human osteocarcoma cell line was also investigated. Phytochemical constituents of both tuber and leaf extract of this plant was studied and analyzed by TLC and GC-MS.

## EXPERIMENTAL SECTION

**Chemicals:** All the chemicals and solvents used in this study were of pure and analytical grade.

### Sample collection and extraction

Taro tubers and leaves were collected from VIT University nursery and were extracted by soxhlet method with methanol for 3-4 hours. The extracted material was later collected and solvent was evaporated under vacuum. The dried sample was stored at 4°C for further experiments.

### Detection of alkaloids

Solvent free 5 mg extract was stirred with few ml of diluted hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents [13].

A. Hager's Test: Filtrate was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow colour precipitate.
B. Wagner's Test: To one ml of filtrate, few drops of Wagner's reagent [ iodine (1.27g) and potassium iodide (92 g) was dissolved in 5 ml of water and made up to 100 ml with distilled water] were added along the side of test tube. Formation of reddish brown precipitate indicates positive test [14].

### Detection of carbohydrates and glycosides

5 mg of leaf extract was dissolved in 5 ml of water and filtered. The filtrate was subjected to the following tests.

A. Fehling's Test: 1 ml of filtrate was boiled on water bath with 1 ml of each of Fehling's solutions A and B. Appearance of red precipitate indicates the presence of sugar.

Fehling's solution A: Copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 ml using distilled water.

Fehling's solution B: Potassium sodium tartarate (173 g) and sodium hydroxide (50g) was dissolved in water and made up to 500 ml.

B. Molish Test: to 2 ml of filtrate, two drops of alcoholic solution of α napthol were added, the mixture was shaken well and 1 ml of conc. sulphuric acid was added slowly along the sides of test tube and allowed to stand for few minutes. A violet ring confirmed the presence of carbohydrates.

**Detection of phytosterols**
Libermann Burchard's Test: The extract (5 mg) was dissolved in 2 ml acetic anhydride. To this, one or two drops of conc. sulphuric acid were added slowly along the sides of the test tube. An array of colour changes indicates the presence of phytosterols [15].

**Detection of phenolic compounds**
Ferric chloride test: The extract (2 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. Appearance of green colour indicates the presence of phenolic compounds [16].

**Detection of flavonoids**
Alkaline Reagent Test: 1 ml of extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, affirms the presence of flavonoids.

Lead acetate Test: 1ml of the plant extract was added in a test tube. To this 1ml of 5% lead acetate was introduced and the mixture was allowed to stand for few minutes. The formation of precipitates in the samples confirmed the presence of flavonoids.

**Detection of Tannins**
About 0.5 g of extract was stirred with about 10 ml of distilled water and then filtered the extract. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Formation of a green or bluish green precipitate indicated the presence of tannins.

**Analytical studies**
**Thin Layer Chromatography**
TLC was done to separate the compounds of taro tuber and leaf methanol extract. Combination of solvents (hexane: acetone; 60:40) was used as mobile phase to separate the compounds according to their mobility. Separated spots were visualized under UV light and Rf factor was calculated by the following equation.

Rf factor = Distance travelled by solute front / Distance travelled by solvent front

**Gas Chromatography – Mass Spectrometry**
Methanol extract of taro tuber and leaves were analyzed by GC-MS. Perkin Elmer Clarus 680 gas chromatographic instrument equipped with a mass spectrometer detector (Clarus 600 model) and an Elite-5MS (30.0 m, 0.25 mmID, 250 µm df) column was used. The carrier gas used was helium at a flow rate of 1 ml $min^{-1}$. The following temperature program was used: initially the oven temperature was held at 60°C for 2 min and then ramped from 10°C/min to 300 °C with hold time for 4 min, total run time 30 min. The temperature of the injector was maintained at 300°C. The ion trap was operated at 70 eV with a scan range of m/z from 50 to 600. A sample of 1 µl was injected in split mode (10:1). The intermediate and end product was identified based on the Wiley registry of mass spectral data [17].

**Antimicrobial studies**
Antimicrobial activity of taro tuber and leaves methanol extract was checked against nine clinical pathogens by agar well diffusion method. Muller-Hinton agar plates were prepared and each plate was inoculated with respective pathogens. 8 mm well was cut on the agar plate surface. Four different concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml) of the extract were added to the well and respective solvent was used as negative control. The plates were incubated for 24 hrs at 37°C. After incubation the plates were observed for zone of inhibition around the well. Antimicrobial activity of tuber and leaves extract was measured by comparing zone of inhibition of extract with negative control [18].

**Antioxidant study**
The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay which was originally described by Blois [19]. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a synthetic free radical with deep violet colour

when is in form of solution with $\lambda_{max}$ at 517nm. It can accept an electron or a hydrogen radical to become stable diamagnetic molecule and appear as light purple in colour which indicates the scavenging of DPPH and the substance has antioxidant activity.

Methanol extracts were prepared for both tuber and leaf extracts. Methanol solution of DPPH was used as negative control. 500µl of each sample and 500 µl of DPPH solution was allowed to react and incubated at room temperature for 30 mins under dark conditions. Absorbance was taken at $\lambda_{max}$ i.e. 517nm against a blank which was 500µl of methanol. Percentage inhibition was calculated by the following equation to conclude the presence of antioxidant activity of the extracts.

Percentage of inhibition = (OD control – OD sample / OD control) x 100

**Anticancer study**

**Cell line**

The human osteosarcoma cell line (MG 63) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% $CO_2$, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

**Cell treatment procedure**

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% $CO_2$, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in dimethylsulfoxide (DMSO) along with aliquot of the sample solution which was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells containing 100 µl of medium, resulting in the required final sample concentrations. Following the addition of sample, the plates were incubated for an additional 48 h at 37°C, 5% $CO_2$, 95% air and 100% relative humidity. The medium without samples was served as control and triplicate was maintained for all concentrations.

**MTT assay**

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader.

The percentage of cell viability was then calculated with respect to control as follows

Percentage of cell viability = [A] Test / [A]control x 100

The percentage of cell inhibition was determined using the following formula.

Percentage of cell inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between percentage of cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software [20, 21].

## RESULTS AND DISCUSSION

**Phytochemical studies**

Phytochemical study revealed the presence of different phytochemical constituents in methanol extract of taro tuber and leaves. The result of phytochemical analysis is presented in table 1. It confirms the presence of alkaloids, flavonoids, carbohydrates, tannins, terpenoids in methanol extract of both tuber and leaves extract. Phytosterols and phenols are observed to be present only in leaves extract and absent in tuber extract. These phytochemicals are

reported to possess different biological activities, such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects [22]. Flavonoids, tannins and alkaloids have hypoglycemic activities [23, 24]. Terpenoids showed the analgesic properties [25].

**Table 1: Phytochemical analysis of *Colocasia esculanta* methanol extract of tuber and leaves**

| Sl no | Phytochemical test | Leaves | Tuber |
|---|---|---|---|
| 1 | Alkaloids | | |
| | Hager's test | + | + |
| | Wagner's test | + | + |
| 2 | Carbohydrates | | |
| | Fehling's test | + | + |
| | Molish Test | + | + |
| 3 | Phytosterols | | |
| | Libermann Burchard's Test | + | - |
| 4 | Phenols | | |
| | Ferric chloride test | + | - |
| 5 | Flavonoids | | |
| | Alkaline Reagent Test | - | - |
| | Lead acetate Test: | + | + |
| 6 | Tannin | + | + |
| | Terpenoids | | |
| 7 | Salkowski test | + | + |

*Note: presence (+) , absence (-)*

**Analytical studies**

**TLC**

Thin layer chromatography was done to separate the components present in methanol extract of taro tuber and leaves by its characteristics Rf values. Two separated spots were observed under UV light using chloroform: methanol (8:2) as mobile phase. Separated spots on TLC plates indicate the presence of different components which were further analyzed by GC-MS.

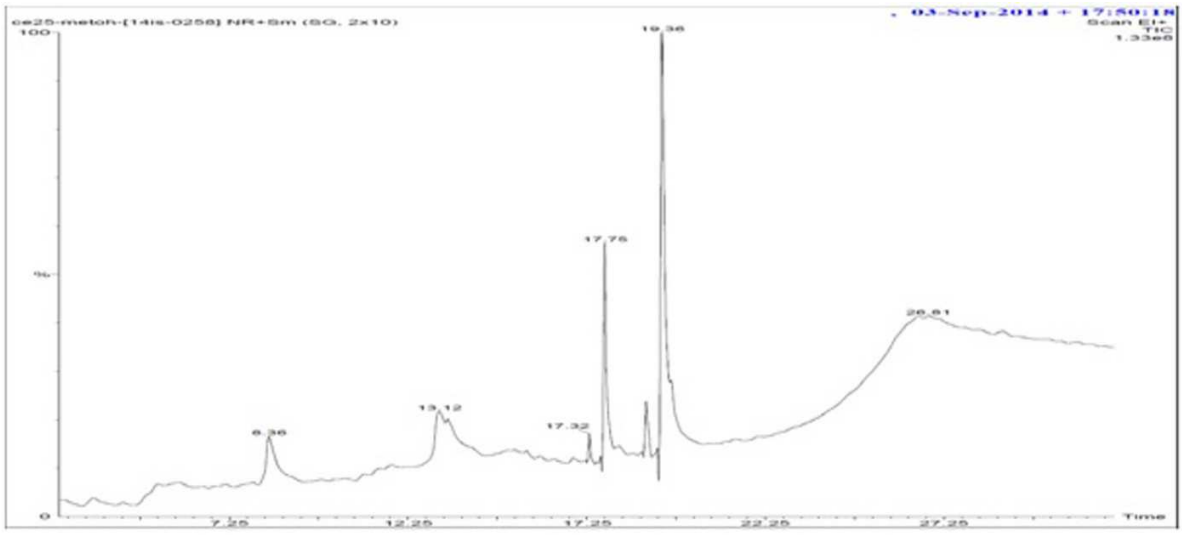


**Figure 1: GC-MS Chromatogram of *Colocasia esculanta* leaves methanol extract**

**GC-MS**

In order to determine the compounds present in the extract of taro tuber and leaves, GC-MS analysis was done. This analysis revealed that methanol extract of tuber and leaves contain different compounds. Some of them are known for their biological activity whereas; activity of few compounds remains unknown. From tuber extract, Decanoic Acid, 10 Fluoro Trimethyl Ester and Pentadecanoic Acid are reported to possess antimicrobial activity [26]. 9,12,15-Octadecatrienoic Acid, (Z,Z,Z)- are reported to reported antioxidant activity by previous workers [27]. N-Hexadecanoic Acid, present in leaf extract are reported to possess antimicrobial and antioxidant activity [28] and

anticancer effect [29]. GC-MS chromatogram of tuber and leaves extract is presented in figure 1 and figure 2 respectively.


**Figure 2: GC-MS Chromatogram of *Colocasia esculanta* tubers methanol extract**

**Antimicrobial study**

Antimicrobial activity of taro tuber and leaves were checked against nine clinical pathogens. Antimicrobial activity was determined by measuring zone of inhibition formed after incubation period. The methanol extract of tuber and leaves showed characteristic zone of inhibition against all pathogens. The zone of inhibition was very pronounced. Highest zone of inhibition was observed at 100mg/ml concentration against *Klebsiella* sp. for tuber extract while leaves extract has shown highest activity at 100mg/ml concentration against *Proteus mirabilis*. In comparison to leaves extract, tuber extract was observed to possess higher antimicrobial activity. Methanol extract of taro tuber and leaves showed antimicrobial activity against both Gram positive and Gram negative [30]. Table 2 and table present the result of the antimicrobial activities of methanol extract of taro tuber and leaves respectively.

**Table 2: Antimicrobial activity of *Colocasia esculanta* tuber methanol extract**

| Pathogens | Concentrations (µl) and Zone of inhibition (cm) | | | |
|---|---|---|---|---|
| | 25mg/ml | 50mg/ml | 75mg/ml | 100mg/ml |
| *Pseudomonas aerugenosa* | 0.6 | 0.8 | 1.4 | 1.6 |
| *Serratia* sp. | 0.6 | 1.00 | 1.2 | 1.5 |
| *Escherichia coli* | 0.7 | 0.8 | 1.4 | 1.6 |
| *Shigella* sp. | 0.8 | 0.9 | 1.9 | 2.2 |
| *Staphylococcus aureus* | 0.6 | 0.8 | 1.4 | 1.6 |
| *Salmonella* sp. | 0.5 | 0.7 | 1.3 | 1.5 |
| *Klebsiella* sp. | 0.6 | 0.9 | 1.3 | 3.00 |
| *Proteus mirabilis* | 0.4 | 0.7 | 1.3 | 1.6 |
| *Enterococcus* sp. | 0.4 | 0.7 | 1.3 | 1.5 |

**Table 3: Antimicrobial activity of *Colocasia esculanta* leaves methanol extract**

| Pathogens | Concentrations (µl) and Zone of inhibition (cm) | | | |
|---|---|---|---|---|
| | 25mg/ml | 50mg/ml | 75mg/ml | 100mg/ml |
| *Pseudomonas aerugenosa* | 0.7 | 1.0 | 1.3 | 1.4 |
| *Serratia* sp. | 0.7 | 0.8 | 1.1 | 1.1 |
| *Escherichia coli* | 0.7 | 1.00 | 1.2 | 1.3 |
| *Shigella* sp. | 0.8 | 1.0 | 1.1 | 1.2 |
| *Staphylococcus aureus* | 0.8 | 1.1 | 1.2 | 1.4 |
| *Salmonella* sp. | 0.7 | 0.8 | 1.0 | 1.2 |
| *Klebsiella* sp. | 0.6 | 0.8 | 1.0 | 1.1 |
| *Enterococcus* sp. | 0.9 | 1.0 | 1.1 | 1.2 |
| *Proteus mirabilis* | 0.6 | 1.0 | 1.2 | 1.5 |

**Antioxidant activity**

The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay which was originally described by Blois [19]. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a synthetic free radical with deep violet colour when is in form of solution which has a $\lambda_{max}$ at 517nm. Methanol extract of taro leaves has shown higher antioxidant activity than tuber extract (table 4). The reducing power of methanol extract indicates presence of some compounds in both tuber and leaves extracts which can donate electron and could react with free radicals to convert them into more stable products and to terminate radical chain reactions. Increased absorbance of reaction mixture indicates increased reducing power of the extract [31].

**Table 4: Antioxidant activity of *Colocasia esculanta* tuber and leaves methanol extract**

| Sl no | sample | Absorbance at 517 nm | % of inhibition |
|---|---|---|---|
| 1 | Standard | 0.823 | |
| 2 | Tuber extract | 0.175 | 78.73% |
| 3 | Leaves extract | 0.150 | 81.77% |

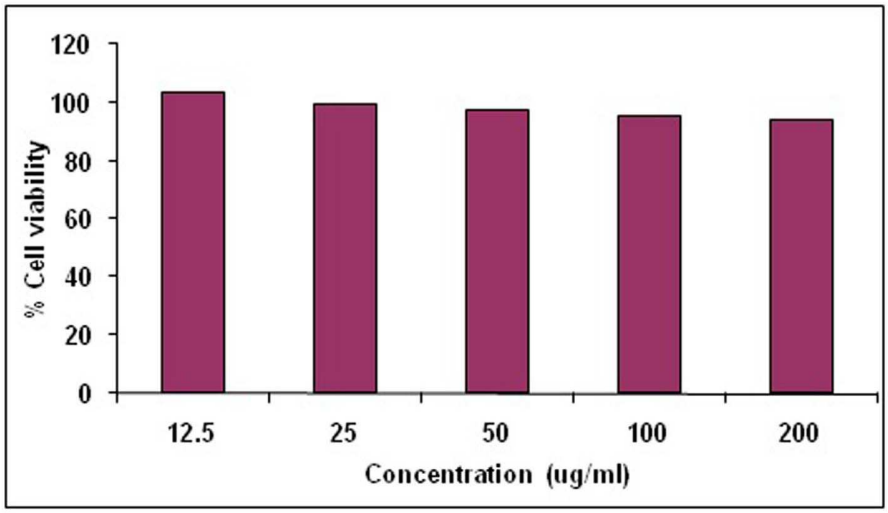


**Fig 3: Anticancer activity of *Colocasia esculanta* tuber extract**

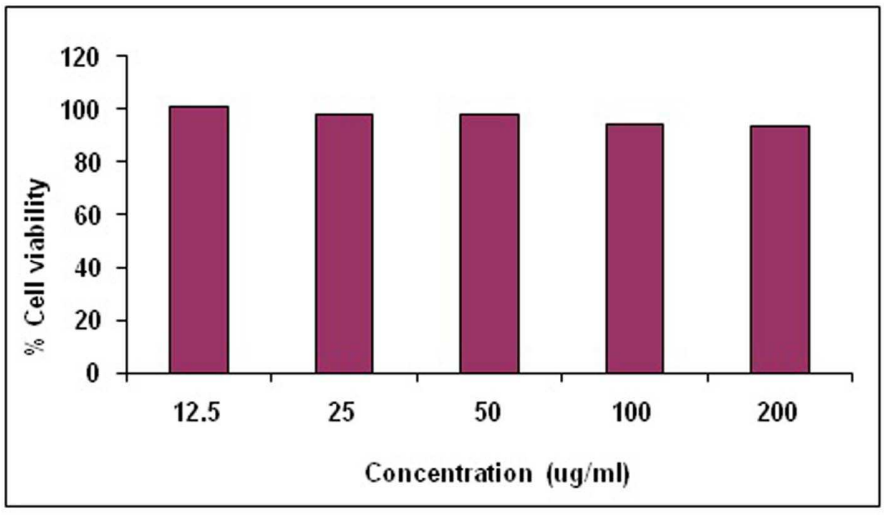


**Fig 4: Anticancer activity of *Colocasia esculanta* leaves extract**

**Anticancer study**

Research into food-derived bioactive components for cancer prevention as well as cancer therapy is growing due to the relatively low or no detectable toxicity and better bioavailability. Anticancer study of the taro tuber and leaves were done against human osteosarcoma cell line (MG 63). Anticancer activity of methanol extract of tuber and leaves were checked. The activity of tuber and leaves methanol extract was presented in figure 3 and figure 4 respectively. In tuber and leaves extract percentage of cell viability is decreased with increased concentration of extract, which indicates the moderate anticancer activity of the extract. Anticancer activity of crude extract of taro against breast cancer was previously reported and tumor cell migration and morphologic changes including cell rounding were observed by previous workers [32].

## CONCLUSION

From the above study, the plant *Colocasia esculanta* is proven to have good antimicrobial, antioxidant and anticancer activities. Bioactivity of *Colocasia esculanta* tuber has projected the great importance of functional foods. As the above result are very promising and encouraging to continue research on *Colocasia esculanta* tubers and leaves and there is a need to purify the compounds to enhance their activity and make them suitable for further clinical approach in near future.

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