

# A Study on Phytochemical Profiling, Antibacterial and Anticancer Activity of *Cordia obliqua* Willd Methanolic Fruit Peel Extract

Saminathan Ponniah<sup>1</sup>, Seetharaman Selvaraj<sup>2</sup>

<sup>1</sup>Director, Sasaam Biologicals Lab Services, Ashok Nagar, Chennai-600083, Tamil Nadu, India

<sup>2</sup>Research Associate, Sasaam Biologicals Lab Services, Ashok Nagar, Chennai-600083, Tamil Nadu, India

Corresponding Author: Saminathan Ponniah

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## ABSTRACT

The purpose of this work is to highlight some of the biological activities of *Cordia obliqua* Willd fruit peel methanolic extract. The phytochemical screening of *C. obliqua* extracts (Chloroform, Methanol and Hexane) was performed; among the three extracts, only methanolic exhibited greater activity. Methanolic fruit peel extracts revealed that the presence of phenol, flavonoids, alkaloids, Saponins and Glycosides. DPPH Assay demonstrated that *C. obliqua* has the highest scavenging activity in methanolic fruit peel extract. The results of antibacterial activity of the tested methanolic fruit peel extract against four pathogenic bacterial strains such as *Salmonella sp.* ( $4.0 \pm 0.141$ ), *Escherichia coli* ( $5.4 \pm 0.223$ ), *Bacillus cereus* ( $7.1 \pm 0.302$ ) and *Staphylococcus aureus* ( $6.1 \pm 0.26$ ). Anticancer activity of *C. obliqua* methanolic fruit peel extract was carried out MDA-MB-231 cell line. At the end of 48 hrs of IC<sub>50</sub> of 269.03 µg/ml, 50% cell viability was observed by following MTT Assay. According to the findings of this study, the active compound found in the *C. obliqua* methanolic fruit peel extract could be a potent source of natural antioxidants, which are important as a therapeutic agent in preventing or slowing the progression of ageing, age-related oxidative stress, and related degenerative diseases. More research is needed to properly characterize the key ingredients responsible for antioxidant and anticancer action.

**Keywords:** *Cordia obliqua*, Antibacterial activity, Antioxidant and Anticancer

## INTRODUCTION

The Boraginaceae family has over 2,700 species that are found in tropical, subtropical, and warmer climates around the world. It is made up of roughly 130 genera and six subfamilies, one of which being Cordioideae. It is home to the genus *Cordia*, which consists of evergreen trees and bushes. Around 300 *Cordia* species have been identified globally. In India, there are 13 species of this genus [1]. In Himachal Pradesh, *Cordia obliqua* Willd. Comes in two varieties, the main distinction between them being the size of the fruits one variety has smaller fruits than the other. The plant with tiny fruits is typically encountered [2]. Its fruit is sweet, contains diuretic, anthelmintic, purgative, expectorant, and maturant properties that make it beneficial for treating dry cough, chest and urethral disorders, biliousness, persistent fever, and joint problems. According to Yunani medicine, it is beneficial for spleen problems. It is beneficial for all lung conditions and is used as a substitute for *Cordia wallichii* in ayurveda [3]. Fruits and vegetables are essentially used food products that can be fully prepared, cooked, or uncooked. It has been discovered that the processing of vegetables and fruits alone results in a significant waste of 25-30% of the overall product. In addition, peels, pomace, rind, and seeds are among the most prevalent wastes. Despite this, the substance

contains beneficial biologically active compounds such as enzymes, carotenoids, lipids, polyphenols, and vitamins. Indeed, these bioactive compounds have showed considerable industrial applications such as food to make edible films, probiotics, and other industrial applications to develop value-added products [4]. It has been observed that considerable amounts of secondary metabolites are present in fruit and vegetable wastes, and extraction has been used to study these waste materials for phenolic compounds, dietary fibres, and other physiologically active metabolites. Thus, fruit and vegetable wastes could be used to produce biologically active metabolites that could be used in the food, cosmetics, pharmaceutical, and textile industries.

Furthermore, the use of innovative components is quite popular within the scientific community as well as the food and pharmaceutical businesses. Fruits contain chemical substances that contribute to unique flavours and attributes, as well as their appearance, nutritional value, and food safety by [5].

Wild fruits are those that have not been domesticated or farmed and instead grow in their natural wild setting [6]. For indigenous populations, wild fruits are key sources of critical nutrients such as dietary fibre, non-

nutritive components, vitamins, and minerals [7, 8]. Current research indicates that several wild fruits can be used to treat cardiovascular problems, type 2 diabetes, urinary tract infections, digestive and inflammatory disorders [9]. The North East area of India, notably Manipur, has an abundance of unique wild fruits and vegetables.

Recently, red dragon fruit has attracted a lot of attention as a natural source of red-purple colour that has a lot of potential for colouring a wide variety of dishes [10, 11]. Furthermore, red dragon fruit is widely known for its high antioxidant activity, which may provide a variety of health benefits [12]. The current study aims to determine phytochemical screening, Antibacterial, antioxidant and anticancer activity (MDA-MB-231) of derived from *Cordia obliqua* methanolic fruit peel extract

## MATERIALS METHODS

### Collection and Identification of Fruit

Fresh fruits *Cordia obliqua* Willd were collected from Kannalam Village (Fig.1), Melmaiyanur taluk, Vilupuram, Tamil Nadu, India, and were identified by Prof. P. Jayaraman, Institute of Herbal Science (IHS), Plant Anatomy Research Centre (PARC), West Tambaram, Chennai, India.



Figure 1: A-*Cordia obliqua* fruits, B- fruit Peel powder

### Preparation of extract

The *Cordia obliqua* fruits were rinsed with deionized water after being washed with tap water. A sharp sterilised knife was used to separate the peels from the fruit. Extracts

were made by soaking 50g of fresh fruit peels in 500 mL of Methanol, Hexane, and Chloroform separately. The infusions were shaken in an orbital shaker for 72 hours. Whatman filter paper 1 was used to filter the

extracts. Solvents were extracted under low pressure. The extracts were then dissolved in known volumes of solvents. For additional investigation, Methanol, Hexane, and Chloroform extracts were used.

#### **Phytochemical analysis**

The fruit peel extracts were submitted to preliminary phytochemical screening using the methodology described by [13, 14, 15].

#### **Bacteria and Fungus culture**

The test organisms were collected from Sasaam Biological Lab Services, Ashok Nagar, Chennai, India. They included gram-positive bacteria as *Bacillus cereus* (NCIM-2217), *Staphylococcus aureus* (NCIM-2079) and gram-negative *Escherichia coli* (NCIM-5662), *Salmonella sp.* (NCIM-2501). The organisms employed are all clinically relevant human pathogenic pathogens.

#### **Antimicrobial activity**

The antibacterial activity of *C. obliqua* fruit peel methanolic extract was determined using the disc diffusion method developed by [16]. In a Petri plate, sterile blank discs were soaked in the sample for roughly 2 hours. The discs were then ready for usage. Discs were placed in MHA agar plates that had been inoculated. After 1 hour of diffusion, the plates were incubated at 37°C for 24 hours. The zone of inhibition was measured using a standard ruler (ZOI). ZOI (Zone of Inhibition) was measured in millimeters. For the positive control test, Ampicillin (10 µg/ml) was employed as an antibiotic.

#### **Determination of zone of inhibition (ZOI)**

After 24 hours of incubation, each plate was examined. A circular inhibitory zone exists on the surface. The diameters of the zones were measured using a ruler to the nearest complete centimeter.

#### **Antioxidant activity of DPPH assay**

The total free radical scavenging ability of methanolic fruit peel extract of *Cordia obliqua* was calculated using by previously reported method [17] with a little modification utilizing the stable DPPH radical, which has an absorption maximum at 515 nm. To make a radical solution,

dissolve 2.4 milligram DPPH in 100 mL methanolic. To 3.995 ml of methanolic DPPH, a test solution (5 µl) was added. The mixture was forcefully mixed and left at room temperature for 30 minutes in the dark. The absorbance of the reaction mixture was measured spectrophotometrically at 515 nm. The absorbance of the DPPH radical in the absence of antioxidant, i.e. the blank, was also measured. All determinations were made in triplicate. The ability to scavenge the DPPH radical was determined using the equation below [18].

$$\text{DPPH Scavenged (\%)} = ((\text{AB}-\text{AA})/\text{AB}) \times 100$$

Where,

AB is absorbance of blank at t= 0 min;

AA is absorbance of the antioxidant at t= 30 min.

A calibration curve was plotted with % DPPH scavenged versus concentration of standard antioxidant (Ascorbic acid).

#### **In Vitro Anticancer Activity [19]**

The cells were grown in the DMEM medium for 6-7 hours at 5% CO<sub>2</sub> at 37°C in a humid incubator. Before introducing the test substance, the cells were harvested, counted (3 × 10<sup>4</sup> cells per mL), and transferred to a 24 well plate. They were then incubated for 24 hours. The test samples were then serially diluted using DMEM medium after the fruit peel extract dissolved in Dimethyl Sulfoxide (DMSO) to achieve the desired concentrations of 62.5, 125, 250, 500, and 1000 µg per mL. The 5 mg/mL MTT solution was mixed in 1 mL of phosphate buffer solution and 10L was added to each of the 24 wells. The wells were wrapped in aluminum foil and incubated at 37°C for 4 hours. Suction was used to remove the solution containing medium, dead cells, and unblend MTT from each well, and 150L of DMSO was added to each well. The fruit peel was then vibrated, and the optical density was measured at 595 nm with DMSO as a blank. For each concentration and cell line, the controls and samples were tested and reproduced. The MTT assay was used to assess the toxicity of the fruit peel extract on cancer cell lines

after incubating mononuclear cells with it for 48 hours. By comparing the absorbance of the control and samples, the toxicity was determined. The values were then used to compute the concentration of fruit peel extract required to cause a 50% decrease (IC<sub>50</sub>) in EAC cell number growth [20].

### Statistical analysis

The data was statistically analyzed, and the Mean + SE for six separate observations were computed. The percentage difference between the Control/Standard and the experiment was determined and displayed in tables. The significance of the sample mean was determined using the student's 'F' test, and differences were judged significant at the p<0.05 level.

## RESULTS AND DISCUSSION

The early phytochemical investigation confirmed the presence of *Cordia obliqua* Peel extracts. The outcomes are listed in the table 1 below. Different solvents revealed different phytochemical classes. They revealed the Presence of tannins, saponins, flavonoids, Alkaloids, Saponins and other compounds. More phytochemical presence in the methanolic fruit peel extract compare to the other fruit peel extract like Hexane and Chloroform. These ingredients may be responsible for the antibacterial, anticancer activity but it is difficult to link their action to a specific phytochemical. These secondary metabolites are reported to have many biological and therapeutic properties [21, 22]. There is excessive amount of phytochemical present in the methanolic fruit peel extract so that the antibacterial, antioxidant and anticancer activity were carryout.

Table 1: Investigation of phytochemical analysis of *C. obliqua* methanolic fruit Peel extracts

S.No	Phytochemicals	Chloroform	Methanolic	Hexane
1	Acid	-	-	-
2	Alkaloids	-	+	+
3	Anthocyanine/Batacyanine	-	+	+
4	Carbohydrates	+	+	-
5	Cardiac Glycosides	-	-	-
6	Coumarines	-	+	-
7	Flavonoids	+	++	++
8	Glycosides	-	+	-
9	Phenols	+	++	-
10	Proteins	-	-	+
11	Quinones	-	+	-
12	Saponins	+	++	-
13	Starch	-	-	-
14	steroids	-	+	-
15	Tannins	+	++	+
16	Terpenoids	+	-	+

+ moderate Present, ++ present and +++ highly present

### Antibacterial activity

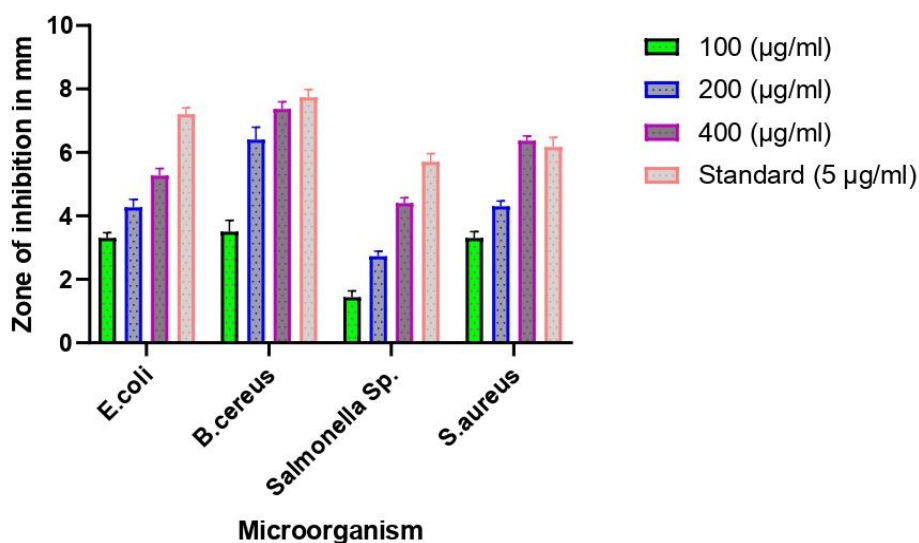
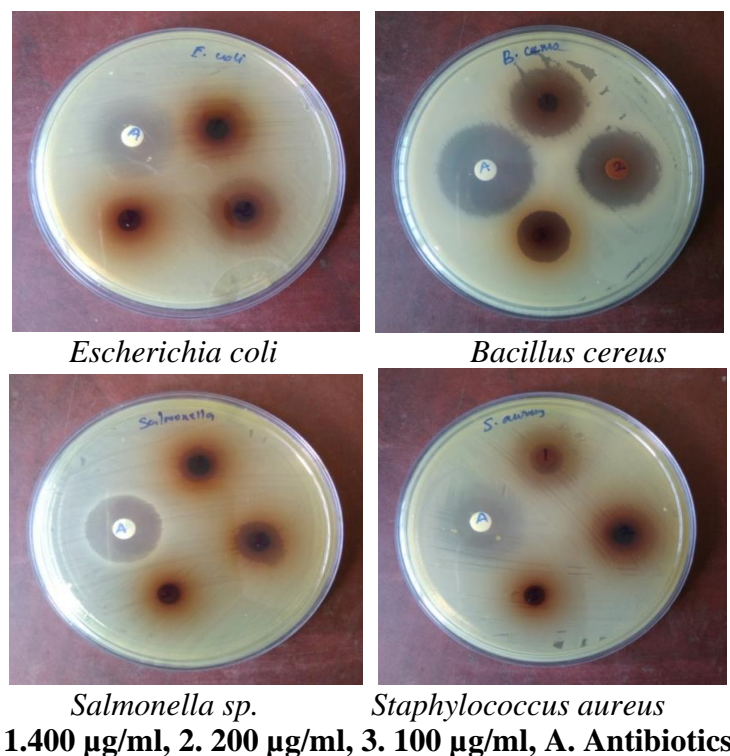
The antibacterial efficacy of *C. blique* methanolic fruit peel extract against therapeutically relevant bacterial pathogens such as *Salmonella sp.*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*, respectively. These four pathogenic bacteria species were also tested with the widely available antibiotic Ampicillin, and the results are shown in figure.2 and table. 2.

The F-test was used to determine the maximum zone of inhibition (MIC) in the methanolic fruit peel extract of *C. blique*, and the differences were found to be significant at the p<0.05 level. Their capacity to combine with extracellular and soluble proteins, as well as the bacterial cell wall, accounts for their activity. Antibacterial activities of flavonoids, phenol, steroids have been reported [23, 24].

Table 2 Antibacterial Activity of methanolic fruit peel extract in different clinical pathogens

S.no	Organisms	100(µg/ml)	200(µg/ml)	400(µg/ml)	Standard (Ampicilin)
1	<i>Escherichia coli</i>	3.2±0.182*	4.3±0.182*	5.4±0.223*	7.4±0.223*
2	<i>Bacillus cereus</i>	3.6±0.22*	6.4±0.266*	7.1±0.302*	7.7±0.273*
3	<i>Salmonella sp.</i>	1.2±0.18*	2.7±0.182*	4.0±0.144*	5.6±0.17*
4	<i>Staphylococcus aureus</i>	3.3±0.182*	4.2±0.111*	6.1±0.26*	5.9±0.170*





Values are expressed in mean ± SEM (n=6); Significant level at the \*- P<0.05

Figure 2: Antibacterial Activity of methanolic fruit peel extract in different clinical bacteria

### Antioxidant activity

Fruit peel methanolic extract showed DPPH radical scavenging activity from *C. blique*. The IC<sub>50</sub> values were determined and are shown in the table 3 and figure 3. All of which were statistically different (p<0.05). According to [25], the ascorbic acid concentration of citron and satkora was 52.35 mg/100 g and 69.29 mg/100 g, respectively, which corresponded to our

findings. To assess the free radical scavenging activity of *C. blique* fruit peel, the DPPH free radical scavenging assay was performed. The DPPH free radical scavenging technique is a widely used method for determining a sample's ability to neutralize free radicals. The DPPH radical scavenging assay measures proton radical scavenging action, which is an important property of antioxidants. Hydrogen donating

ability of the antioxidants molecules contributes to its free radical scavenging nature.

The IC<sub>50</sub> value of *C. blique* methanolic fruit peel extract and L-Ascorbic acid was 104.92(μg/ml) and 23.85 (μg/ml)

respectively. The results show that *C. blique* methanolic fruit peel extract has a high inhibitory potential against stable free radicals. This study reveals that *C. blique* fruit peel extract has higher scavenging activity and L-Ascorbic acid.

Table 3: DPPH Assay of IC<sub>50</sub> values of the respective extract and Ascorbic Acid.

S.No	Concentration (μg/ml)	Methanolic fruit peel extract	L-Ascorbic acid (Standard)
1	1000	20.52±0.35* (-79.69)	9.73±0.18* (-90.26)
2	500	29.30±0.21* (70.69)	19.55±0.28* (-80.44)
3	250	33.75±0.26* (-66.84)	36.22±0.23* (-63.77)
4	125	35.75±0.365* (-64.26)	52.56±0.285* (-47.44)
5	62.5	56.16±0.294* (-43.83)	67.65±0.236* (-32.34)
6	31.25	72.19±0.44* (-27.80)	76.73±0.53* (-23.5)
	IC <sub>50</sub>	104.92(μg/ml)	23.85 (μg/ml)

Values are expressed in mean ± SEM (n=6); Significant level at the \* - P<0.05

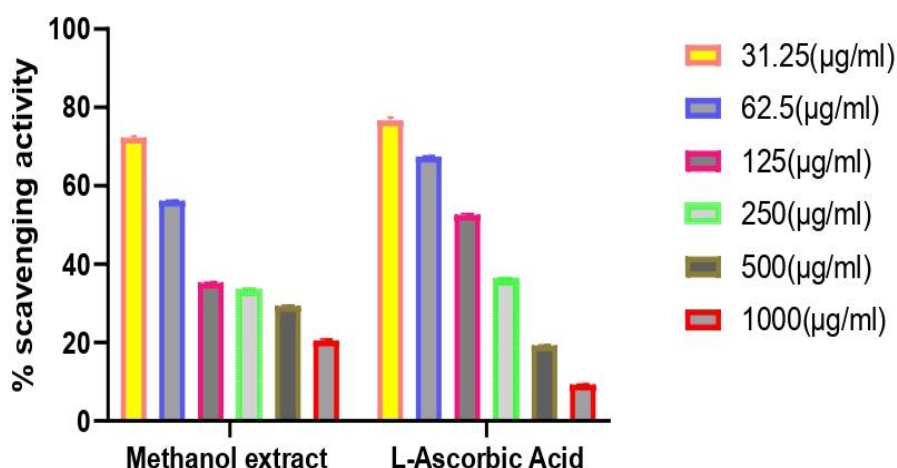


Figure 3: DPPH Assay of IC<sub>50</sub> values of extract and Ascorbic Acid.

### Anticancer activity

Table 4 shows the effect of fruit peel methanolic extract of *C. blique* the MTT assay on the MDA-MB-231 cell line. In a dose-dependent way, the concentration of extract increased the percentage cytotoxicity and decreased cell viability in cell lines. Table 4 and figure. 4 show the possible anticancer action of fruit peel methanolic extract on MDA-MB-231 cell lines. Fruit peel methanolic extract of *C. blique* had this strongest activity against MDA-MB-231 cell line. Fruit peel methanolic extract produced results that were comparable to Control.

Despite major advances in chemotherapeutic drugs, cancer-related morbidity and death remain serious concerns. However, preventative agents are scarce and of poor utility [26]. *C. blique* of fruit peel extract was found to have high antioxidant and free-radical scavenging activities in this investigation, which may be responsible for the anticancer impact. Excessive free radical production causes lipid peroxidation, which can lead to tissue deterioration and oxidative stress. [27] SOD activity is inhibited as a result of tumor development. SOD and CAT are free-radical scavengers found in oxygen-

metabolizing cells that protect against the detrimental effects of superoxide and hydrogen peroxide. [28] Fruit peel extract reduced lipid peroxidation and restored antioxidant enzyme (GSH, SOD, and CAT) activity, indicating MECD's antioxidant and free radical scavenging properties. [29, 30] investigated the mechanism of action and signal pathways of *Psidium guajava* peel extract in killing prostate cancer cells (MCF-7). *C. blique* of fruit peel extract demonstrated good antioxidant and free-

radical scavenging activities, which can cause cancer cells to die. Apoptosis is a frequent method of action for chemotherapeutic medicines, including natural compounds derived from fruit and plants. The induction of it is critical to the efficacy of fruit-derived natural compounds as anticancer treatments. [31] *C. blique* of fruit peel extract has the ability to specifically cause apoptosis in cancer cells, showing its anticancer efficacy.

Table 4: shows the effect of fruit peel methanolic extract of *C. blique* the MTT assay on the MDA-MB-231 cell line.

S.No	Concentration (µg/ml)	Methanolic fruit peel extract
	Control	100
1	1000	26.54±0.50 (-73.45)*
2	500	36.16±0.52 (-63.83)*
3	250	51.13±0.46 (-48.86)*
4	125	58.20±0.47 (-41.86)*
5	62.5	70.62±0.27 (-29.37)*
	IC <sub>50</sub>	269.03(µg/ml)

Values are expressed in mean ± SEM (n=6); Significant level at the \*- P<0.05

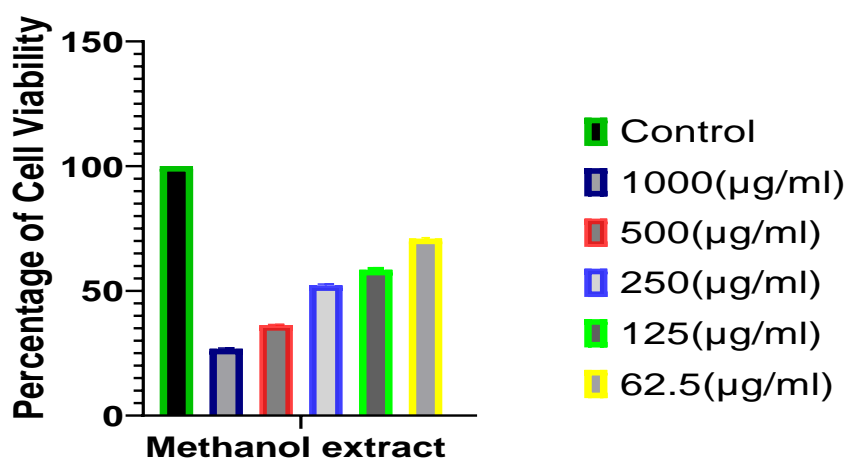


Figure 4: shows the effect of fruit peel methanolic extract of *C. blique* the MTT assay on the MDA-MB-231 cell line.

### Cell morphology Studies of MDA-MB-231

Morphological Investigation of Cells (MTT Assay) Even at modest dosages, treatment of MDA-MB-231 cells with these *C. blique* fruit peel methanolic extract caused morphological changes in the cells, which had a comparable effect on cell morphology. After treatment with the

extracts (1000, 500, 250, 125 and 62.5µg/ml), most of the cells turned spherical in form and were not adhered to the substrate in a dose-dependent manner (Figure. 5). Based on the findings, we may conclude that *C. blique* extracts may have influenced cell-cycle and activated apoptotic pathways.

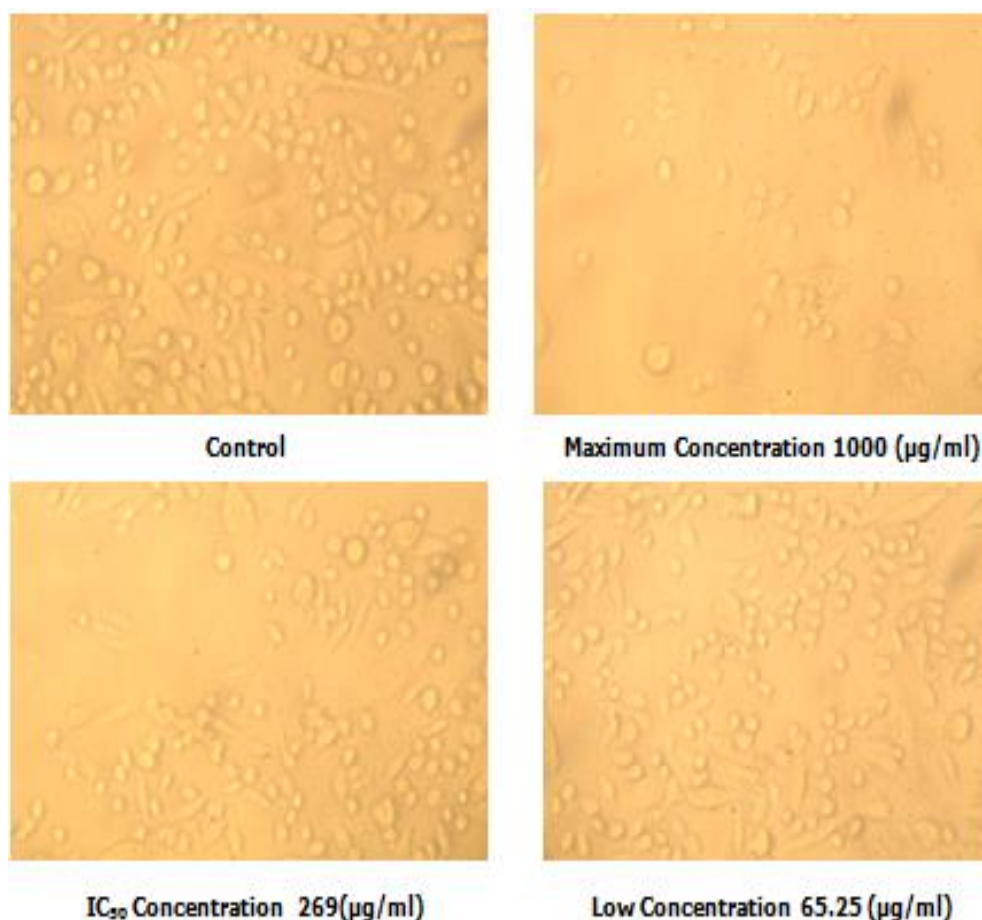


Figure 5: Cell morphology of MDA-MB-231 cells when treated with IC<sub>50</sub> concentrations of fruits peel methanolic extract of *C. blique*

## CONCLUSION

In conclusion, an in vitro study of the methanolic extract of *C. blique* fruit peel revealed potential antibacterial, antioxidant and anticancer effects against human MDA-MB-231 cancer cells. The identification of potent phytoconstituents in the extracts will serve as a natural cytotoxic agent against many cancers, and increasing knowledge, promotion, and usage of this fruit for public benefits is strongly urged.

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