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# Antimicrobial and Anti-cancer Activity of Capsicum *chinense* Bhut Jolokia fruit: An *In-vitro* Analysis

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## Abstract

The present study was aimed to estimate the phytochemical components and antimicrobial activity of Capsicum *chinense* Bhut Jolokia fruit extracts. In this investigation, the fruit extracts contained more numbers of phytochemical constituents and potent antimicrobial activity against pathogenic microorganisms. In addition it lays the ground for the new research that is testing new varieties of Capsicum for cytotoxic studies. The lung cancer (A549) cell lines were used to screen the in vitro anticancer challenging with the fruit extracts. Concentration ranges from 6.25 to 100 µg of fruit purified extracts were screened against (A549) cells. The anticancer activity of fruit extract was performed by 3-[4, 5–Di methyl thiazol-2-yl]-2, 5-diphenyltetrazolium Bromide (MTT) assay. The cytotoxicity was highly significant in the 100 µg concentration of fruit extract in the lung cancer (A549) cell lines. These results concluded that Capsicum *chinense* Bhut Jolokia fruit have good antimicrobial and cytotoxic effect which serve as an important source of pharmacological applications

**Keywords:** Capsicum *chinense* Bhut Jolokia fruit, phytochemicals, antimicrobial activity, MTT assay

## Introduction

Capsicum an herbaceous plant belongs to the family *Solanaceae*. Bhut jolokia belongs to the species Capsicum *Chinense* Jaqc. Characteristic pungency and flavour of the chili pepper fruits

provide unique hotness to meal preparations and a wide variety of foods utilize this pungency in combination with salt and other spices for flavor enhancement (Pino *et al.*, 2007). In addition to the use of chili pepper as a spice, it has been widely used as an agent to preserve food (Omolo *et al*., 2014). The indigenous uses of chili pepper in folk medicinal applications and the safe and prolonged use of chili pepper as a spice in human history enable us to think that the phytochemicals present in chili pepper fruits promote and maintain the good health of human beings (Bosland, 1996).

The phenolic compounds present in the chili pepper are attributed to many medicinally important properties such as anti-diarrheal, antimicrobial, antioxidant, antihyperglycemic, anti-lithogenic and antimutagenic activities (Adefegha and Oboh, 2013). Generally chili pepper has a higher amount of vitamin C (ascorbic acid) than many other vegetables showing its significant nutritional value (Kumar and Tata, 2009). Treating hypersensitivity and rheumatism using chili pepper also was common in Italian indigenous medical practices (Pieroni *et al*., 2004). Capsaicin is also known to induce the apoptosis pathway in leukemia cells through oxidative stress (Ito *et al*., 2004). (Lee *et al*., 2010) also reported that capsaicin represses the cell proliferation in colorectal cancer cell lines. Herbal materials have long been appreciated because of their proven antimicrobial effects (Alavijeh *et al*., 2012). Therefore the present study deals with the screening of phytochemical compounds, antimicrobial activity and cytotoxic effect on cancer cells.

## Materials and Methods

### Plant sample

Capsicum *chinense* Bhut Jolokia (Solanaceae) fruit was obtained from a cultivated area in karungal, Kanyakumari District, Tamil Nadu, India. The seeds were removed from a fresh fruit sample. The fruit without seeds and the seeds were left to dry, separately, in a circulating air oven (40 °C) for seven days and then triturated to powders. To obtain the ethanolic extract the plant material (1 kg of fruits or 0.2 kg of seeds) was extracted with 70% ethanol (plant: solvent, 1:10, w/v) under reflux for 4 h. The fruit and seeds of Capsicum *chinense* (5 kg of fruits or 1 kg of seeds) were also submitted, separately, to successive extractions in a soxhlet apparatus using chloroform, *n*-butanol and water until complete exhaustion, in order to obtain the chloroform, butanol and aqueous extracts, respectively. The organic solvents were evaporated under reduced

pressure to dryness and, the aqueous extract was lyophilized, to obtain the respective residues from fruit and seeds.

## Qualitative analysis of phytochemical constituents

All the extracts were subjected to systematic phytochemical screening for testing the presence of various phytochemical constituents by the standard protocols by (Harborne *et al*., 1973). Phytochemical test includes carbohydrates, amino acids, proteins, vitamin C, chloride, tannin, alkaloids, flavonoids, phlobatannins, steroids, phenols and saponins.

## Antimicrobial activity of plant extracts

Antimicrobial activities of the fruit extracts were determined by well diffusion method by (Anushia *et al*., 2009). Four bacterial pathogens such as *Escherichia coli, Klebsiella pneumonia, Bacillus subtilis,* and *Staphyloccocus aureus* and three fungal pathogens such as *Aspergilus niger, Aspergillus fumigatus* and *Penicillium* sp. were used for this investigation. The test bacterial strains were inoculated into Nutrient broth and incubated at 37°C for 24 h. After the incubation period, the culture tubes were compared with the turbidity standard. Fungal inoculums were prepared by suspending the spores of fungus (as previously cultured) in saline water mixed thoroughly, made turbidity standard and used.

Fresh bacterial culture of 0.1 ml having 108 CFU was spread on Muller Hinton agar plates using sterile cotton swabs. The fungal strains were spread on Potato dextrose agar. Wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50 µl of plant extracts using micro pipette in aseptic condition. The plates were kept in a refrigerator to allow pre-diffusion of extract for 30 min and then incubated at 37°C for 24 h and 28-30 37°C for 3-4 days for bacterial and fungal cultures respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

## MTT assay

MTT assay measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by the enzyme mitochondrial succinate dehydrogenase. The MTT enters into the cells and passes to the mitochondria and it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then treated with an organic solvent Dimethyl

sulfoxide (Himedia) and the released product was measured at 540nm. Since reduction of MTT can occur only in metabolically active cells the level of activity is the measure of the viability of the cells. The cells was then washed with 1x PBS and then added 30 µl of MTT solution (MTT -5mg/ml dissolved in PBS). It was then incubated at $37^0$C for 3 hours. MTT was removed by washing with 1x PBS and 200 µl of DMSO was added to the culture. Then it was incubated at room temperature for 30 minutes until the colour was developed. The solution was then transferred to centrifuge tubes and centrifuged for 2minutes to precipitate the cell debris. Optical density was read at 540 nm using DMSO as blank in a micro plate reader (ELISASCAN, ERBA).

## Results

### Qualitative analysis of phytochemical constituents

Phytochemical screening of the fruit extracts was performed for the analysis of phytochemical constituents In the leaf material, aqueous extract showed positive results for carbohydrate, chloride, tannins, alkaloids, flavonoids, Phlobatannins, steroids, phenolic compounds and saponins; Chloroform extract showed positive results only for chloride, tannins, saponins and phenolic compounds; Butanol extract showed positive results for chloride, tannins and phenolic compounds (Table 1).

**Table 1. Qualitative analysis of phytochemical constituents**

| Sl. No. | Chemical constituents | Fruit extracts | | |
|---|---|---|---|---|
| | | Aqueous | Chloroform | Butanol |
| 1 | Carbohydrates | + | - | + |
| 2 | Protein | - | - | - |
| 3 | Amino acid | + | + | + |
| 4 | Vitamin C | + | - | - |
| 5 | Chloride | - | - | - |
| 6 | Tannins | + | + | + |
| 7 | Alkaloids | + | + | + |
| 8 | Flavonoids | + | + | + |
| 9 | Phlobatannins | - | - | + |
| 10 | Steroids | - | - | + |
| 11 | Phenolic compounds | - | + | - |
| 12 | Saponins | - | + | + |

'+' presence of compound; '-'absence of compound

## Antimicrobial activity of fruit extracts

Antimicrobial activity of the fruit extracts were determined by well diffusion method against various bacterial and fungal pathogens. The aqueous extract showed inhibition activity on *K. pneumonia* (10 mm), *S. aureus* (16 mm), *B. cereus* (18 mm) and *A. fumigatus* (20 mm); chloroform extract showed activity on *E. coli* (10 mm), *S. aureus* (12 mm), *Penicillium sp* (10 mm); and butanol extract showed activity on *E. coli* (10 mm), *S. aureus* (11 mm), *A.niger* (10 mm) and *Penicillium sp* (16 mm) (Table 2).

**Table 2. Antimicrobial activity of Capsicum *chinense* Bhut Jolokia fruit extracts**

| Sl. No. | Test organisms | Leaf | | |
|---|---|---|---|---|
| | | Aqueous | Chloroform | Butanol |
| 1. | *E.coli* | - | 10mm | 10mm |
| 2. | *K. pneumonia* | 10mm | - | - |
| 3. | *S. aureus* | 16 | 12mm | 11mm |
| 4. | *B. cereus* | 18 | - | - |
| 5. | *A. fumigates* | 20 | - | - |
| 6. | *A. niger* | - | - | 10mm |
| 7. | *P. chrysogenum* | - | 10 | 16mm |

## Zone of inhibition in ‘mm’

## Anticancer activity

The anticancer effect of Capsicum *chinense* Bhut Jolokia fruit extract was determined using MTT assay. The present finding indicated the percentage inhibition of A549 cell lines (Fig.1). The (Fig.2) displayed the anticancer activity of fruit extracts of Capsicum *chinense* Bhut Jolokia in A549 cells. The aqueous extract illustrates higher cytotoxicity in 100 µg concentrations. Finally, the present study suggests that the aqueous extract of Capsicum *chinense* Bhut Jolokia fruit comprised novel anticancer compounds which will be potent therapeutic agent for lung cancers.

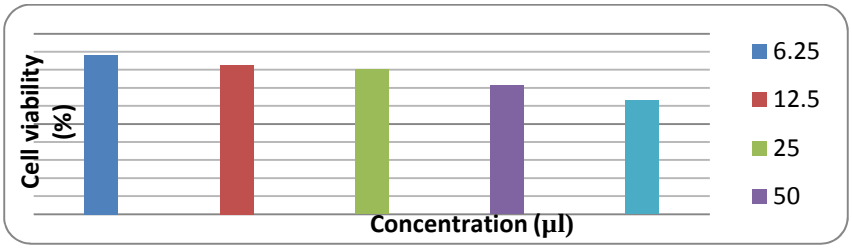
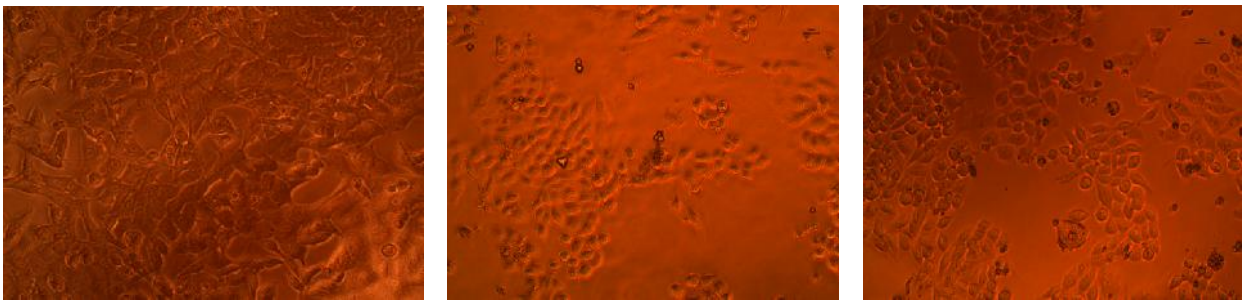
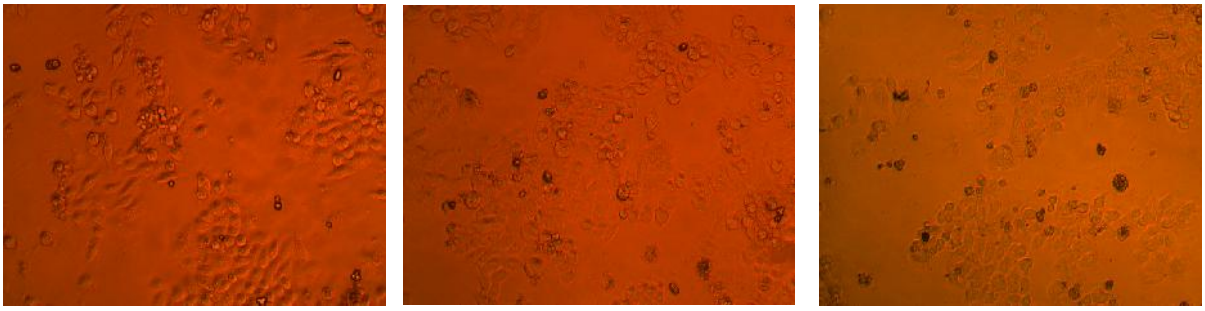



**Fig. 1: Percentage viability of A549 cell lines after the exposure with the fruit extract of Capsicum *chinense* Bhut Jolokia**



**Control** **cell viability at 6.25µl Concentration** **cell viability at 12.5µl concentration**



**Cell viability at 25µl Concentration** **Cell viability at 50µl Concentration** **Cell viability at 100µl Concentration**

**Fig. 2: Anticancer effect of Capsicum *chinense* Bhut Jolokia fruit extract on lung cancer cell lines**

## Discussion

The aqueous extracts of Capsicum *chinense* Bhut Jolokia fruits showed wide antimicrobial activities against the pathogens. Similarly in the present study Capsicum *chinense* Bhut Jolokia were screened for the anticancer activities against lung cancer. The A549 cells were used to study the anticancer activities. In this Capsicum *chinense* Bhut Jolokia fruit extract showed potent anti-cancer activity. The phytochemicals present in chili pepper possess anticancer properties (Chung *et al*., 2005). Application of C. *chinense* fruit extracts to the HepG2 cell lines (derived from a hepatocellular carcinoma) demonstrated the inhibition of cancer cell proliferation. This inhibitory activity was independently verified by using methylthiazol tetrazolium (MTT), lactate dehydrogenase leakage and nitrous oxide (NO) production assays (Amruthraj *et al*., 2014). The studies on human cell cultures with exogenous capsaicin exhibited

apoptosis (Ghosh and Basu, 2010), autophagy (Choi *et al*., 2009; Oh *et al*., 2010) and inhibition of cell metabolism (Arora *et al*., 2011). Based on the antibacterial action and anti-cancer activity it can be concluded that phytochemicals present in Capsicum *chinense* Bhut Jolokia fruits is very effective in the prevention of a lot of disease.

## Conclusion

From this preliminary investigation it has been concluded that the Capsicum *chinense* Bhut Jolokia fruits having significant antimicrobial and anti-cancer activity, the phytochemical compounds present in the Capsicum *chinense* Bhut Jolokia fruits might be a responsible active constituent for this activity. Further research is in progress to isolate the compound responsible for this activity.

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