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Research Article

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Anticancer Potential of Operculina Turpethum in MCF-7 Human Breast Cancer Cell Lines

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ABSTRACT

Objective: This study aims to evaluate the anticancer potential of Operculina turpethum toward MCF-7 human breast cancer cell lines.
Materials and methods: Different concentrations of ethanol extract and choloroform extract of Operculina turpethum roots were investigated for their anticancer potential in vitro through MTT assay in MCF-7 human breast cell lines.
Results: The ethanol extract and the chloroform extracts of Operculina turpethum showed dose dependent inhibition of cell growth. Out of the two extracts chloroform extracts showed highest inhibition when compared with ethanolicextracts of Operculina turpethum.
Conclusion: the plant Operculina turpethum showed excellent cytotoxicity activity against MCF-7 cells, hence it can be used in the development of newer anticancer drugs.

Keywords: Operculina turpethum; MCF- 7; MTT; Breast cancer; Percentage inhibition l

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INTRODUCTION

Cancer become one of the major globan burden and increasing in delvelping countries [1]. Every year the incidence of cancer cases just keep escalating and do not seem to go down. It is well-known as one of the leading causes of death worldwide.

In Indian women, breast cancer was found more common and it is prevalent in urban women when compared with rural women. A survey reports that one lakh new breast cancer patients were diagnosed annually [2]. Indian Council of Medical Research (ICMR) reports that the number of breast cancer cases in India to rise to 106,124 in 2015 and to 123,634 in 2020 [3].

People who are overweight have a greater chance of developing breast cancer. The prevalence of breast cancer in Indian women is more at the age of forty [4]. But early diagnosis may result in complete cure, patients in the late stage have to undergo series of treatment plan such as chemotherapy and surgical intervention. This cocktail of treatment methods and drugs is highly toxic and has multiple side effects. Thus needing other prevention-related or nonconventional therapeutic strategies. Hence, there is a need for better options for therapy and prevention of the disease. A survey by World Health Organization states that around 80% of the overall population across the world rely on phytomedicine and 33% of drugs used are from plant sources [5] and almost 3000 species of plants are currently being used in cancer therapy [6].

One such plant with anticancer potential is *Operculina turpethum* (L.) Silva Mansowhich is from Convolvulaceaefamily [7]. The species has common names such as Indian Jalapandand Turpeth. In Tamil,

it is commonly called as Karunchivadai. The plant species is rich in various kinds of alkaloids, such as coumarins, turpethin, α and β rhamnose, fructose, scopletin, β-sitosterol, betulin and lupeol. Various researchers report that *Operculina turpethum* having multiple pharmacological actions such as hepatoprotective [8], antimicrobial [9], antiulcer [10], antidiarrhoeal [11], anti-arthritic [12], analgesic [13] and antidiabetic activity [14].

The anticancer activity of *Operculina turpethum* was not studied so far. Hence an attempt have been taken to find the effect of *Operculina turpethum* against the breast cancer cell line MCF-7 (Figure 1).

Figure 1: ***Operculina turpethum***

MATERIALS AND METHODS

Collection and Authentication

The plant, *Operculina turpethum* was collected from rural belt of Tirupati, Andhra Pradesh and was authenticated by Dr. K. MadavaChetty from Sri Venkateswara University, Tirupati. The roots and rhizome of the plant was collected in bulk andwashed with tap water to remove the soil and dirtparticles and then shade dried. The dried plantmaterials were milled into coarse powder.

Preparation of the Extracts

The powdered plant root materials (250 g) were successively extractedby coldmaceration process using solvents of increasing polarity namely, chloroform and ethanol, each for three days. Then the crude extracts was filtered and concentrated over a rotary evaporator and further dried in a vacuum oven to get constant weight (Peach and Tracey, 1955). The extracts were stored in desiccators.

Materials

MCF-7 cells purchased from National Center for cell line sciences (NCCS), Pune
MTT,(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) - HiMedia, Mumbai
Fetal bovine serum- GIBCO
Trypsin-HiMedia, Mumbai
Penicillin-HiMedia, Mumbai
DMSO- FISCHER, India
DMEM (Dulbecco's Modified Eagle's medium)-HiMedia, Mumbai
CO_2 incubator- Thermo Fisher, USA
Multimode microplate reader- Perkin Elmer
Refrigerated centrifuge- Eppendorf Germany

Procedure

5×10^5 MCF-7 cells were seeded in 96 well plates. Cells were allowed to attach to the plates by incubation at 37ºC overnight in a CO_2 incubator. The cells were then exposed to ethanolic extracts and chloroform extracts of *Operculina turpethum* at various concentrations (10, 20, 40, 60, 80 and 100 µg/ml) and incubated for 48 hours at 37°C. MTT solution was added to the plate at a final concentration of 5 mg/mL and incubated for 4 hr in dark at 37°C. The resulting MTT product was dissolved by DMSO. Cell Viability was calculated by measuring optical density at 650 nm using ELISA reader. Cells were incubated with

MTT dye for 3 hours in dark at 37°C. The resulting MTT product was dissolved by DMSO. The absorbance were read at 570 nm in a microplate reader. All the results of the MTT Assay were analyzed using Graph Pad Prism 5.1

RESULTS

The percentage inhibition at various concentration for the ethanolicextract of *Operculina turpethum and* chloroform extract of *Operculina turpethum* were noted and tabulated (Tables 1 and 2). Tables 1 and 2 showed the percentage of inhibition produced was dose dependent.

The ethanolic root extract of *Operculina turpethum* showed the lowest percentage inhibition of 34 ± 0.47 at 10 µg/ml, whereas at 100 µg/ml showed the maximum percentage inhibition of 81.2 ± 0.13. From the Table 1, it was clear the activity produced by ethanolic root extract of *Operculina turpethum* was dose dependent. The effect of various concentrations of ethanolic extracts on percentage inhibiton was shown in Figure 2. The IC_{50} value was found to be 32.50 µg /ml.

The chloroform root extract of *Operculina turpethum showed the lowest percentage inhibition of* 34.2 ± 0.32 at 10 µg/ml, whereas at 100 µg/ml showed the maximum percentage inhibition of 85.5 ± 0.10. From the Table 1, it was clear the activity produced by ethanolic root extract of *Operculina turpethum* was dose dependent. The effect of various concentrations of choloroform extracts on percentage inhibiton was shown in Figure 3. The IC_{50} value was found to be 16.25 µg/ml.

The effect of ethanolic extracts and cholorform extracts of *Operculina turpethum*on percentage inhibition on MCF-7 cells were given in Figure 4.

Table 1: The anti-cancer activity of the ethanolic root extract of *Operculina turpethum* by MTT assay on MCF-7 cell line

Concentration (µg/ml)	% Inhibition
10	34.0 ± 0.47
20	39.0 ± 0.24
40	60.9 ± 0.34
60	66.2 ± 0.40
80	68.4 ± 0.33
100	81.2 ± 0.13

Table 2: The Anti-cancer activity of the chloroform root extract of *Operculina turpethum*by MTT assay on MCF-7 cell line

Concentration (µg/ml)	% Inhibition
10	34.2 ± 0.32
20	55.7 ± 0.23
40	71.4 ± 0.20
60	78.1 ± 0.02
80	79.0 ± 0.11
100	85.5 ± 0.10

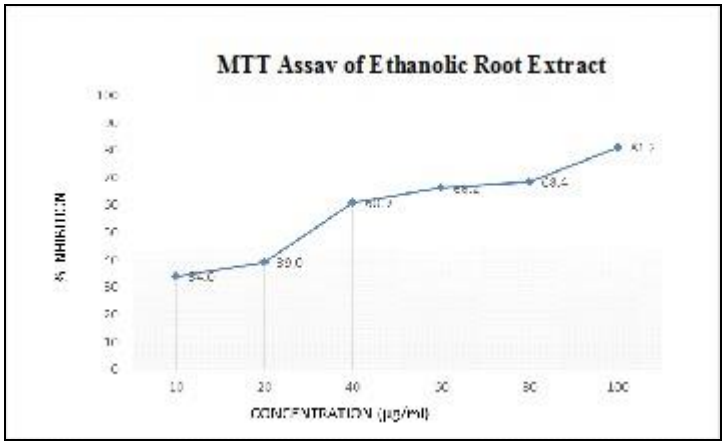

Figure 2: MTT assay of the ethanolicroot extract of *Operculina turpethum*. Values are expressed in mean ± S.D in triplicates

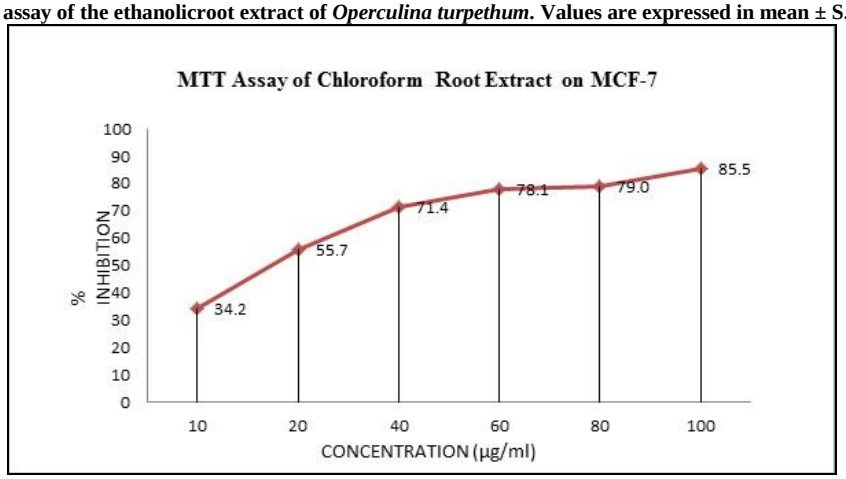

Figure 3: MTT assay of the chloroform root extract of *Operculina turpethum*

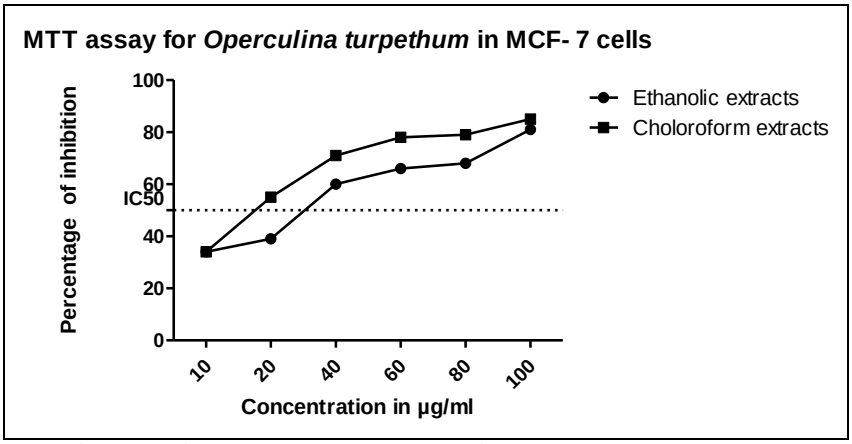

Figure 4: MTT assay of the chloroform and ethanolicroot extract of *Operculina turpethum*. Values are expressed in mean ± S.D in triplicates

From the above, it is clearly evident that the chloroform extract has shown significant anticancer activity with an IC_{50} of 16.25 µg/ml, whereas the ethanolic extract has an IC_{50}value of 32.50 µg/ml respectively.

DISCUSSION

Being one of the major causes of death around the world, cancer is fast becoming a ghastly disease. Various observations showed that there is an increase in resistance towards anticancer drugs. This resulted in a very bad situation in the treatment of cancer. Nowadays alternative medicine from natural products are developed to cure cancer. Many natural plants have furnished modern medicine with the drugs that are used in cancer therapy as cytotoxic agents [15-17]. This has led to research on natural products that have the ability to inhibit cell proliferation and reduce the spread of cancer. The phytochemical analysis of the choloroform extract of the plant reveals that the plant has components such as alkaloids, triterpenoids and flavonoid, It was observed that the chloroform extract of the had high anticancer activity than the ethanol extract in MCF- 7 cell lines.

CONCLUSION

Chloroform root extract of *Operculinaturpethum*was considered to have a high anticancer potential compared to ethanolic root extract of *Operculina turpethum.* In future, we also plan to isolate the major compound responsible for anticancer activity and further to find the mechanism of the anticancerous potential of the plant, in particular, we will investigate whether the plant extract influences DNA methylation and gene expression in breast cancer.

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