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Antiproliferative effect of methanolic extract of *Mallotus philippensis* in MCF-7 cell lines

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ABSTRACT

Phytochemicals and their derivatives are promising options for improving cancer treatment efficiency while minimising side effects. Methanolic extract of flowers of *Mallotus philippensis* were assessed for their cytotoxicity in MCF-7cell line by 3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay at concentrations of 640, 320, 160, 80, 40, 20, 10 and 5 µg/mL and the half maximal inhibitory concentration (IC50) was calculated using Graph Pad Prism 5.0. As a positive control, doxorubicin was used. Phytochemical analysis of *M. philippensis* methanolic extract using standard tests revealed the presence of alkaloids, tannins, flavonoids, and phenolic compounds. When the cells were exposed to different concentrations of the extracts, a dose-dependent reduction in cell viability was observed. The IC_{50} of *M. philippensis* methanolic extract is 41.28 µg/mL. The antiproliferative effect of methanolic extract of *M. philippensis* in cancer cells in a dose dependent manner could be a promising strategy in chemotherapy. This extrapolates the use of natural products in drug designing conducting clinical trials in vivo and in human subjects.

Keywords: Cancer, *Mallotus philippensis*, MCF-7 Cell lines, MTT Assay.

INTRODUCTION

Cancer is one among the most deadly disease present throughout the world. It is just not a single disease; but a group of disease that destroy the normal body tissue. The development of abnormal tissue in one organ and its infiltration and metastasis to other organs leading to the destruction of normal cells made the disease "the second leading cause of death in the world" [1,2]. The rising incidence of various types of cancer necessitates the development of novel anticancer drugs. The conventionally used anticancer drugs could not only destroy the abnormal cells but also attacks the viable cells [3]. Thus, the need of the hour is to develop a new molecule which could destroy the abnormal cancer cells while not harming the normal cells in body. In this scenario, phytochemicals and their derivatives may be promising options for improving cancer treatment efficacy with fewer side effects. Plants are being used since ancient times as medicine. Recently, the anticancer researches are based on the cytotoxic activities of plant natural products. The antitumor activity of a novel compound can be assessed by its antiproliferative effect, alteration of antigens on tumor cells and stimulation of fatal differentiation of altered cells [4]. MCF-7 cell line is a commonly used cancer line for studying the cytotoxic activities *in vitro*. These cell lines are positive for estrogen and progesterone receptors and negative for human epidermal growth factor receptor [5].

Mallotus philippensis, a member of the Euphorbiaceae family, was widely used in traditional medicine, particularly in tropical areas. It is found in the Himalaya from Kashmir eastwards, as well as tropical India [6]. It is also known as kamala, kampillaka, kapila, and locally as shendri. Previous research has shown that the plant has anti-helminthic, antiphrastic, antifilarial, antifungal, antiulcer, antibacterial, and immuno-regulatory properties, as well as being an aphrodisiac [7,8]. *M. philippensis* is an excellent source of medicinally significant natural molecules, and its potential use in modern medicine is promising. Even though the leaves of *M. philippensis* are tested for cytotoxicity, there is no evidence of cytotoxic activity in the flower. As a result, the current study assessed the antiproliferative effect of a methanolic extract of *M. philippensis* flowers.

MATERIAL AND METHODS

Plant material

The flowers of *M. philippensis* flower were procured from the Wayanad District of Kerala state, India. The flowers were cleaned and dried in shade, powdered to a coarse form using an electric pulverizer, and the extraction was done using methanol as solvent in Soxhlet hot extraction method. The extract was

was then loaded into a rotating vacuum evaporator for further concentration. After evaporating methanol, the extract was stored in an air tight container at 4°C.

Phytochemical Screening

The qualitative phytochemical analysis assessed the presence of various constituents like flavonoids, alkaloids, phenols, steroids, terpenoids, saponins, tannins, etc. using standard procedures as described by [9].

Cell line

The National Centre of Cell Science in Pune provided the MCF-7 human breast cancer cell lines. After that, it was cultured in tissue culture flasks with Dulbecco's Modified Eagle Medium (DMEM) containing 10% foetal bovine serum and 1% gentamicin (50 mg/mL). The cells were incubated in a CO_2 incubator set to 37°C.

Cytotoxicity studies: MTT (3-(4,5- dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay

According to [10], the *in vitro* cytotoxic potential of the methanolic extract of *M. philippensis* flowers was evaluated in MCF-7 cell lines using the MTT reduction assay. The cells were uniformly cultured in a microtiter plate (96 wells) at a concentration of 5×10^4 cells/mL and then incubated for 24 hours for maximum active cell growth. The cells were treated with diluted extract at concentrations of 640, 320, 160, 80, 40, 20, 10, and 5 µg/mL. As a positive control, doxorubicin was used. After 24 hours, 10 µL of MTT at a concentration of 5mg/mL was added to each well and incubated for 4 hours with 100 µL of serum free medium. To stop the reaction, 100 µL of DMSO was added. At a wavelength of 594nm, the absorbance is measured using an enzyme-linked immunosorbent assay. The percentages of cell viability and inhibition were calculated as follows.

% cell viability = (Average absorbance of treated cells /Average absorbance of untreated cells) % cell inhibition = % cell viability.

Using graph pad prism v.5.0, the IC_{50} values of extracts were calculated by plotting concentration versus percent cell inhibition.

RESULT

Qualitative Phytochemical Analysis

The flower extract contained phenolics, alkaloids, tannins, glycosides, terpnenes, and flavonoids, according to the results of the qualitative phytochemical analysis. The result of phytochemical analysis is summarized in table 1.

Cytotoxic evaluation of methanol extract of *M. philippensis* in MCF-7 cell line:

The MTT assay results revealed a dose dependent increase in the percent cell inhibition, with cells treated with 640µg/mL showing the greatest inhibition. At 640µg/mL, the maximum inhibition was 76.52%. Table 2 shows the percentage inhibition of cell proliferation as measured by the MTT assay 24 hours after treatment with methanolic extract in the MCF7 cell line. Table 3 shows the percentage of cell viability after 24 hours of treatment with methanolic extract. The IC_{50} value of the methanolic extract of *M. philippensis* flower as determined by the MTT assay was 44.28 µg/mL. The IC_{50} obtained is shown in figure 1.

Table 1: Phytochemical constituents present in the methanolic extract of *Mallotus philippensis*

Phytochemical constituents	Methanolic extract of *M. philippensis*
Alkaloids	+
Terpenes	+
Glycosides	+
Phenolic compounds	+
Saponins	-
Flavonoids	+
Steroids	-
Tannins	+
"+" indicate the presence and "–" indicate the absence of phytochemical	

Table 2: The per cent cell inhibition of MCF-7 cells after 24 hours treatment with methanolic extract of *M. phillipensis*

Concentrations of extract (µg/mL)	% cell inhibition
640	76.52
320	71.43
160	63.11
80	69.99
40	63.33
20	47.63
10	46.15
5	39.76

Table 3: The per cent cell viability of MCF-7 cells after 24 hours treatment with methanolic extract of *M. Phillipensis*

Concentrations of extract (µg/mL)	% cell viability
640	23.48
320	28.57
160	36.89
80	30.01
40	36.67
20	52.37
10	53.85
5	60.24

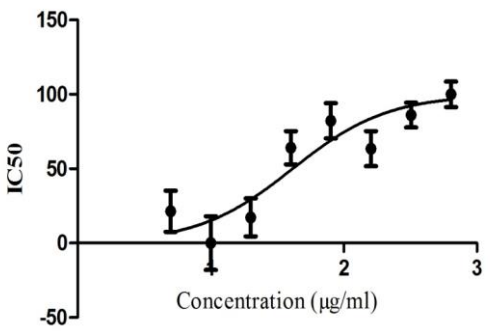

Figure 1: IC_{50} (µg/mL) for Methanolic extract of *M. phillipensis* is 44.28 µg/mL

DISCUSSION

Breast cancer is an invasive type of cancer that primarily affects women. It is one of the most common types of cancer and the second leading cause of cancer-related death in women. Age, gender, inheritance, mutations, smoking, food, menopause, obesity, and radiation exposure have all been identified as risk factors for breast cancer [11]. MCF-7 cell lines are the best model for assessing the cytotoxicity potential of any lead molecule for the therapy of breast cancer. The MTT assay can be used to assess the cytotoxicity of various phytochemicals on live cells. Live cells in this assay convert the chemical MTT to purple formazan crystals, whose colour is measured spectrophotometrically [12]. The methanolic extract of *M. philippensis* reduced cell viability in a dose-dependent manner in the current study. As the extract dose increased, cell viability decreased. The lowest cell viability was measured at a dose rate of 640 µg/mL. The present study also indicated that the extract work by the mechanism of antiproliferation of cancer cells which is one among the three mechanisms targeted by the anticancer therapeutics. However, the MTT assay cannot detect and the extent of apoptotic ability of the cells and also to differentiate necrosis and apoptosis as the mechanism for cell reduction, for which further studies are needed [13]. The phytochemical analysis revealed the presence of various secondary metabolites in the extract. The presence of such compounds may be the reason for the antiproliferative effect of the extract. Phenolic compounds and flavonoids were shown to exhibit anticancer properties [14].

CONCLUSION

The current study found that *M. philippensis* methanolic flower extract had significant antiproliferative activity in MCF-7 cell lines in a dose-dependent manner. As a result, the extract has the potential to be developed as a promising lead molecule in the development of anticancer drugs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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