SHORT COMMUNICATION

Anticancer activity of *Ziziphus mauritiana* roots against human breast cancer cell line

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Abstract: This study was designed to evaluate the anticancer activity of *Ziziphus mauritiana* roots. The dichloromethane and methanol extracts were prepared and anticancer activity was investigated the by using MTT assay. Human breast cancer cell line (MCF-7) was used in this study. 50µg/ml of dichloromethane extract of the roots of plant exhibited significant anticancer activity (70%) against the breast cancer cell line with IC_{50} 20.34±0.9 using doxorubicin as standard. The study indicated that *Ziziphus mauritiana* has anticancer activity against MCF-7 cell line.

Keywords: *Ziziphus mauritiana,* human breast cancer cell line, MCF-7

INTRODUCTION

Cancer is characterized by an unregulated cell growth. The most common types of cancers being lung cancer, stomach cancer, liver cancer, colorectal cancer and breast cancer (WHO, 2010). Globally, cancer is among the leading causes of death. Although various advancements in the prevention and treatment of cancer have occurred, the successful treatment remains a challenge. Chemotherapy is a common treatment that has proved useful in a number of cancer cases including breast, colorectal, pancreatic, osteogenic sarcoma, testicular, ovarian and certain lung cancers. However, poor selectivity and toxicity limits the use of chemotherapy. Therefore, the advancements in identifying new drugs with higher selectivity and less toxicity have gained momentum. Consequently, the exploration of herbal therapies to identify novel drugs has increased.

Ziziphus mauritiana belongs to the family Rhamnaceae. The family comprise of 58 genera and 900 species. Many species of the genus *Ziziphus* are found in Pakistan, Iran, Europe, America, India and Afghanistan. The plant *Ziziphus mauritiana* is commonly known as Berry, as "Ber" in India and "Chotta Ber" in Pakistan (Ashraf *et al.*, 2015). Plant is enriched with biological and phytochemical properties. The plant is used to cure wide variety of diseases such as ulcer, asthma, depression, and anxiety (Cisse *et al.*, 2009). The various species of genus *Ziziphus* showed antipyretic, anti-inflammatory and analgesic properties (Nisar *et al.*, 2007; Adzu *et al.*, 2016). The *Ziziphus mauritiana* also showed antioxidant activity and antimicrobial activity (Ashraf *et al.*, 2015).

The genus *Ziziphus* has many phytochemical constituents, cyclopeptide alkaloids such as Abssenine B, Zazyphine D, Amphibine C, Oxyphylline A and also saccharides like rhamnose and arabinose (Gournelis *et al.*, 1997). Cancer management is one of the top hot issues among medicinal research today.

The evaluation of anticancer activity of *Ziziphus mauriitiana* root extracts against human breast cancer (MCF-7) cell line was the objective of the present study.

MATERIALS AND METHODS

Plant collection

The roots of *Ziziphus mauritiana* was collected from surroundings of Bahauddin Zakariya University in 2015 and identified by Associate Professor Dr. Zafrulla Ullah Zafar, Plant Taxonomist, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan, where a voucher specimen No. STEWART-469(6) was deposited.

Extraction

The freshly collected roots of *Ziziphus mauritiana* (100 g) were taken and shade dried, ground and extracted successively with the solvents (3x6L) at room temperature for 24 hours. Two solvents from the opposite pool, methanol (Polarity index 3.1) and dichloromethane (Polarity index 5.1), were used to extract a wide range of constituents. The extracts were concentrated under vacuum on Rota vapor model No. Buchi-rotavapor R.200. The dichloromethane extract was obtained brown crude extract (1.64mg) while methanol extract was obtained as dark brown crude extract (13.37g).

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Table 1: Anticancer activity of dichloromethane and methanolic extracts of *Ziziphus mauritiana* roots

	Concentration	% Inhibition	IC_{50} ± SD [µg/ml]
Dichloromethane extracts	50 µg/ml	70	20.34 ±0.9
Methanolic extracts	50 µg/ml	12	-
Standard (Doxorubicin)	50 µg/ml	89	0.92 ±0.1

Anticancer activity (MTT assay)

Cytotoxic activity of compounds was carried out by using standard colorimetric assay. Cell cultured were prepared and spray was carried out into 96 micro plates. In every well, 100µl of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in phosphate buffered saline was added and incubated at 37°C for 4hr. The medium with MTT was flipped off and the formed formazancrystals was solubilized in 100µl of DMSO. Using micro plate reader, the absorbance was measured at 490nm. The % cell inhibition was determined using the following formula:

$$\% \text{ Cell inhibition} = 1 - \frac{\text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

STATISTICAL ANALYSIS

Microsoft Excel 2013 was used to measure logistic linear regression and $p<0.05$ was considered as significant.

RESULTS

The dichloromethane and methanol extracts were prepared and anticancer activity was investigated by using MTT assay. Human breast cancer cell line (MCF-7) was used in this study. The dichloromethane extract of roots of the plant showed significant anticancer activity with IC_{50} 20.34±0.9 using doxorubicin as standard.

According to the American National Cancer Institute (NCI), the criteria of cytotoxic activity for the crude extracts is an IC_{50} <30µg/ml. The dichloromethane extract of the plant exhibited significant anticancer activity with IC_{50} value of 20.34±0.92µg/ml which falls within the NCI criteria, thus it is considered as promising anticancer potential.

DISCUSSION

Many plants have recently be reported with anticancer activity like *Casuarina equisetifolia* (Shafiq *et al.*, 2014), *Aspergillus niger* (Channabasava *et al.*, 2014), *Morus nigra* (Qadir *et al.*, 2014) and *Ruellia squarrosa* (Afzal *et al.*, 2015). In this study, MTT analysis showed the anti-proliferative activity of dichloromethane extract of *Ziziphus mauritiana* roots. Dichloromethane extract of *Ziziphus mauritiana* roots showed 70% inhibition of MCF-7 cell proliferation at a dose of 50µg/ml. Flavonoids are the very important constituents of *Ziziphus* species and their anticancer activity has been confirmed previously (Kandaswami *et al.*, 2005). Therefore, the flavonoids in *Ziziphus mauritiana* roots might be involved in anticancer activity.

CONCLUSION

The findings of this work support the use of *Ziziphus mauritiana* roots for the development of natural pharmaceuticals to be useful in the treatment of human breast cancer.

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