

## The Effects of *Emex spinosa* Extracts on PC3 and HeLa Cell Lines

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### Abstract

#### Background

Plants having therapeutic roles have been scrutinized and used from start of human history. *Emex spinosa* is commonly known as Devil's thorn. *Emex spinosa* found in different parts of Pakistan particularly Chakwal, Khuashab and Thal desert. *Emex spinosa* is considered mysterious medicinal plant and traditionally used as purgative, appetizer, antispasmodic and diuretic.

#### Objective

The present research study was planned to evaluate the cytotoxic potential of *Emex spinosa* (Polygonaceae).

#### Methods

The cytotoxic effects of dichloromethane and methanol extracts are investigated against Hela and PC3 cell lines by following MTT assay in which Doxorubicin used as standard drug for comparative purposes

#### Results

Dichloromethane and methanol extract of *Emex spinosa* exhibited various activities including antioxidant, antimicrobial, antispasmodic, anti-inflammatory and cytotoxicity. The results of MTT assay described that dichloromethane extract showed 75.2 % inhibition with  $22.5 \pm 0.15$  IC<sub>50</sub> on PC3 cell lines while methanol extract showed inhibition 63.2 % with IC<sub>50</sub>  $26.4 \pm 1.5$  on HeLa cell lines.

#### Conclusion

The current work reveals that *Emex spinosa* contains various classes of phytochemicals and possessed cytotoxic effects. The promising findings of this research could be used as novel anticancer drug against PC3 and Hela cells.

**Key words:** *Emex spinosa*, Cytotoxicity, MTT assay, Hela cell line, PC3 cell line.

### Introduction

Cancer put a solemn burden on the health of public and its cure and therapeutic procedure are still challenging scientifically. The treatment of cancer includes following main conventional approaches chemotherapy, radiotherapy and surgery. But all these approaches have severe side effects. (Md Islam et al. (2018). Globally the fourth most frequent cancer among females is cervical cancer. The pharmacologic and therapeutic properties exhibited by medicinal plants are generally ascribed to the possession of constituents known as secondary metabolites.

*Emex spinosa* is an important member of family Polygonaceae. It is annual herb and is inhabitant of Mediterranean region. *Emex spinosa* germinates in autumn-winter and can merely reproduce by seed. (Shaltout, El-Beheiry, Ismail, and Ahmed (2009). Among medicinal plants *Emex spinosa* has great importance and is used to cure stomach disorders, dyspepsia and relief colic. It also acts as appetite stimulant. It may consider as diuretic and purgative. (Donia, Soliman, El Sakhawy, Yusufoglu, and Zaghloul (2014).

### Problem Statement

*Emex spinosa* have no clear substantiation of medicinal properties. At present, pharmacological data of *Emex spinosa* is very limited to link folk medicinal uses of it. A continuous demand exists in favor of new therapeutically agents to prevent and cure this disease. The majority of the cytotoxic drugs is not selective and has an effect on dividing cells as well as normal cells; by this means these agents may cause increased harm risks and systematic toxicity. Accordingly, requires the development of antineoplastic drugs which bare minimum side effects, less toxicity and are economical.

### Justification/Scope of Study

Globally there is focus on plants for exploring of novel medicinal alternatives used to treat various cancers. Pakistan has great biodiversity and there are many native plants giving potential lead molecules which can be used in development of new drugs. It is also necessary to evaluate safety risks associated to consumption of all those constituents. As a result this will lead to provision of safe and economic substitutes in the treatment of cancer. These studies will also definitely attract the pharmaceutical industries for exploring the importance of medicinal plants.

### Objectives

The definite objective of this research study was

To evaluate the extracts of whole plant *Emex spinosa* for their anticancer activities.

### Literature Review

In an investigational study the phytochemical analysis of different parts of *Emex spinosa* plant confirmed the presence of phenols, alkaloids, phytosterols, triterpenoids and flavonoids, whereas sugars and glycosides were found in only leaves. Methanol extract was used in different tests for detection of various phytochemicals. In case of stem, the total phenolic content measured 77.84 mg/g with  $\pm 0.32$  standard deviation comparatively less than fruit that is 97.09 mg/g having standard deviation value  $\pm 0.18$ . The total phenolic content of leaf is 97.7 mg/g. The method used for quantifying Total flavonoids was  $\text{AlCl}_3$  colorimetric. Leaf, stem and fruit contained total flavonoids content 58.66 mg/g, 15.08 mg/g and 37.39 mg/g respectively with standard deviation value  $\pm 0.38$ ,  $\pm 0.28$ , and  $\pm 0.12$  in that order. (S. Jan et al. ).

A study conducted to analyzes the antioxidant potential of three natural medicinal plants belonging to Bahrain, namely *Emex spinosa*, *Aizoon canariense* L., and third plant

*Asphodelus tenuifolius*. Free phenolics content measured as mg/100g and *E. spinosa* contained the highest value (64.64) then *A. tenuifolius* (45.21) and least value (32.23) of *A. canariense*. Highest total flavonoids demonstrated by *Emex spinosa* i.e. 82.71 mg/100 g after that 55.92 mg/100g *A. canariense* followed by *A. tenuifolius* (49.1). (Al-Laith et al. (2015).

Nutritive potential of *Emex spinosa* was measured in a study which demonstrated that the metabolic constituents found in this plant are moisture content, total fat, ash, crude protein, total carbohydrates and fiber were 6.4%, 1.1%, 11.45, 19.05% 41.50 % and 20.50%, respectively. Aerial parts of *Emex spinosa* owned the nutritive value 253.9 Kcal/100g. Methanol and water extracts were analyzed for estimation of secondary metabolites. These metabolites were higher in quantity in methanolic extracts as compared to aqueous extracts. All estimated secondary constituents were higher in methanol extract of *E. spinosa* than that of aqueous extract, excluding saponins which were higher in water extract. Methanol extracts yield 6.61 % total phenol content while aqueous extracts yield 8.76 %. The alkaloids were 0.84% in aqueous extract and about 0.55 % in methanol extract. The flavonoids were estimated 1.05% in extracts of methanol while 1.48% in water extracts. But saponins content (0.56%) measured as higher in aqueous extract of *E. spinosa* as compared to methanolic extract 0.56%. ( Abu Ziada et al. (2015).

The analysis of ethyl acetate extracts of *Emex spinosa* (aerial parts as well as underground parts) by using GC-MS showed existence of several biochemicals and some fatty acids including palmitic acid & linoleic acid. ***Capric acid also found in underground fraction of ethyl acetate extract*** ( Makni et al. (2018).

Freije et al. (2013) stated that *Emex spinosa* owned considerable quantity of omega-3 fatty acids, linoleic acid and

omega-6 fatty acids. These fatty acids were extracted from leaves of this plant suggesting that *Emex spinosa* has significant pharmacological as well as nutritional value and can be used in treatment of different diseases.

A study conducted to determine the cytotoxicity effects of *Emex spinosa*, the extracts of leaf, fruit and stem was used. The cytotoxicity activity was determined against brine shrimps and followed the method described by Atta-ur-Rehman & co researchers in 2001. In-vitro results revealed that methanol stem extract of dose 50 and 500ug/ml has prominent effect while on the other hand 5ug/ml has less significant effect. The lethality percent at 5ug/ml was 20 %, 50ug/ml was 33.3 % and 46.6 % at 500ug/ml and showed LD50 value of 52ug/ml. Leaf methanol extract exhibited the lethality percent at 5ug/ml was 33.3 %, 10ug/ml was 73.3 % and 80 % at 500ug/ml and 20ug/ml LD50 value. The methanol extract of fruit of *Emex spinosa* also showed lethality 66.6 % at 500ug/ml and 47ug/ml LD50 value. (S. Jan et al. 2014). A research study conducted by Donia et al. (2014) evaluated bioactive contents, antibacterial and cytotoxic potential of *Emex spinosa*.

### Traditional Uses of *Emex spinosa*

*Emex spinosa*, a historical herb has been reported in several studies as traditional herb. It has been used in pain management, dyspepsia, jaundice, liver problems, indigestion as purgative.

### Research Methodology

The research study was performed at laboratories of Pharmaceutical Chemistry department, faculty of Pharmacy, Bahauddin Zakariya University Multan, Pakistan

### Plant Collection & Extraction

Mature whole plants were uprooted and placed in collection bags. This plant collection was done in March 2020 from sanday areas of tehsil Chobara district Layyah. The whole

plant (leaves, stems, roots) after drying was subjected to extraction process by using dichloromethane and methanol solvents followed by screening for phytochemical constituents present in this plant as well as therapeutic evaluation. Extraction was performed and as result dichloromethane &

Methanol extract was prepared and stored in small jars. These extracts were labeled as ESWPD and ESWPM respectively.

The cytotoxicity of methanol and dichloromethane extracts of *Emex spinosa* was determined for two selected cancer cell lines namely Hela & PC3 cell lines by applying MTT assay performed at HEJ.

S :No	Traditional use	Reference
1	Pain Killer	Abd El-Kader et al. (2006)
2	Dyspepsia	Soliman et al. (2012)
3	Jaundice	El-Hilaly, Hmammouchi, and Lyoussi (2003)
4	Liver problems	Al-Saleh, Ali, and Mirza (1993)
5	Indigestion	Al-Saleh et al. (1993)
6	Purgative	Rahman, Mossa, Al-Said, and Al-Yahya (2004)
7	Diuretic	Watt and Breyer-Brandwijk (1962)
8	Stimulant appetite	Rahman et al. (2004)
9	Biliousness	Rahman et al. (2004)
10	Anti-inflammatory	Abd El-Kader et al. (2006) .

**Table: 1 Traditional uses of *Emex spinosa***

## Results

### Results of Cytotoxic Activity

In this study cytotoxicity of methanol and dichloromethane extracts of *Emex spinosa* was determined against two human cell lines; Hela and PC3. The inhibition of cell proliferation exhibited by Methanol extract for Hela cell line at 30µg/L was

found to be 63.2 % with an IC<sub>50</sub> value of 26.4 ± 1.5 µg/ml. The inhibition of cell proliferation exhibited by dichloromethane extract for Hela cell line at 30µg/L was found to be 18.2 %. The inhibition of cell proliferation with Standard Doxorubicin at concentration 30µg/L was 101.2% with an IC<sub>50</sub> value of 0.9 ± 0.14 for Hela cell line.

The inhibition of cell proliferation exhibited by Methanol extract for PC3 cell line at 30 $\mu$ g/L was found to be just 5.1 % and found to be inactive. The inhibition of cell proliferation exhibited by dichloromethane extract for PC3 cell line at

30 $\mu$ g/L was found to be 75.2% with an IC<sub>50</sub> value of 22.4  $\pm$ 0.15  $\mu$ g/ml. The inhibition of cell proliferation with Standard Doxorubicin at concentration 30 $\mu$ g/L was 80.9% with an IC<sub>50</sub> value of 1.9  $\pm$  0.08 for PC3.

Sample	Test conc. ( $\mu$ g/ L)	Hela		PC3	
		% cytotoxicity	IC 50	% cytotoxicity	IC 50
ESWPM	30	63.2	26.4 $\pm$ 1.5	5.1	inactive
ESWPD	30	18.2		75.2	22.5 $\pm$ 0.15
DOXORUBICIN std	30	101.2	0.9 $\pm$ 0.14	80.9	1.9 $\pm$ 0.08

**Table: - 2 MTT Assay results**

### Discussion

It was established in few earlier research studies that *Emex spinosa* plant possessed cytotoxic activity. The current study specifically determined anticancer activity on PC3 and HeLa cells of human. In a previous study Donia et al. (2014) Aleo-modin glucoside was isolated which proved to be active cytotoxic agent from *Emex spinosa*. This compound showed noticeable anticancer activity against Hep G<sub>2</sub> and Hep<sub>3</sub> B cell lines. The present study was conducted to determine the cytotoxic activity of *Emex spinosa* extracts on two important cancer cell lines i.e. PC3 and HeLa cell line. The result showed that Dichloromethane extract exhibited pronounced activity against PC3 cell line while the methanol extract showed prominent activity against HeLa cell line.

### Conclusion

The current work reveals that *Emex spinosa* contains various classes of phytochemicals like alkaloids, anthraquinones, saponins and phytosterols and possessed cytotoxic effects. The promising findings of this research could be used as novel

anticancer drug against PC3 and Hela cells.

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