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Research Article

**EVALUATION OF CYTOTOXIC ACTIVITY OF
DICHOLOROMETHANE (DCM) AND METHANOL (MEOH) EXTRACT
OF ROOT BARK OF *CAPPARIS DECIDUA*****Muhammad Kamran Khan^{1*}, Muhammad Uzair², Talal Shaheer¹, Zeeshan Javaid¹, Fahim
ullah², Muhammad Taufiq Ahmad¹, Qazi Amir Ijaz¹**¹Akson college of Pharmacy, Mirpur University of Science and Technology AJ&K Pakistan²Faculty of Pharmacy, Bahauddin Zakarya University Multan Pakistan**Abstract:**

The present study was conducted to evaluate the cytotoxic activity of different extracts of root bark of Capparis decidua (Capparidaceae). The root barks of Capparis decidua were collected from village Kirri khaisore Tehsil Pahar pur, Dera Ismail Khan; shade dried and ground into fine powder.

The weighed plant material was then extracted with dichloromethane and methanol in triplicate for 72 hours by the process of successive maceration. The filtrate obtained was concentrated under reduced pressure at 30 °C using rotary evaporator.

Brine shrimp lethality bioassays and MTT colorimetric assay were carried out on the extracts to assess the cytotoxic activity. Dichloromethane extract showed moderate brine shrimp lethality activity, while this activity was not shown by methanol extract. Both extract showed no cytotoxic activity.

Keywords: *Capparis decidua, cytotoxicity, Brine Shrimp*

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INTRODUCTION:

The plant *Capparis decidua* also known as *Capparis aphylla* is large, spinous shrub, climbing, densely branched, thorny shrub or tree. The height of plant is 6 – 10 meter. Plants have a clear bole of the 2-4 meter. The leaves are present only on the young shoots. Life span of leaves is short. Leaves are linear, simple, pointed and a very small. Leaves are sessile or with a short petioles. In November – January new leaves grow. Flowers are red or pink and rarely yellow. The profuse flowering occur on the old shoots however new shoots bear a few flowers. The season of flowers is Feb – July however peak flowering appear in a summer. The fruits are small, glabrous and beaked at apex. The green fruit appear to start in the March- April and 2nd flash of a fruit appear in the May- July. The mature fruit occur before the onset of monsoon. *Capparis decidua* plant shows the top root system, initially primary root is developed which is then produced secondary branches. The roots can be penetrating up to a four meter in case of a pant maturity. Stem of plant is slender, spinous and smooth. Most stems are glossy and dark green in color [1]

The plant *Capparis decidua* grows abundantly in arid, dry and exposed habitat like drying pools, ditches, and wasteland, road side and surrounding plane of the hill [2]. The plant is widely distributed in, Arabia, India and Pakistan [3].

Various studies have been conducted to assess the biological and pharmacological activities of *Capparis decidua*. Results of these studies concluded that the this plant possess anti-diabetic activity [4], Anti-atherosclerotic activity [5], Anthelmintic activity [6], Antibacterial activity [4], Hypolipidaemic activity [7], Analgesic, Anti-Inflammatory and CNS Depressant Activity (Navanath, Naikwade, & Mule, 2009), Hypercholesterolemic activity [8] and hepatoprotective activity [9].

However, literature suggests that no study has been performed to evaluate the cytotoxic activity of Dichloromethane (DCM) and methanol (MeOH) extracts of root bark of *Capparis decidua*. The present study aims to evaluate the cytotoxic activity of Dicholoromethane (DCM) and Methanol (MeOH) extract of root bark of *Capparis decidua*.

MATERIALS AND METHOD:

Collection of Root barks of *Capparis decidua*

Root barks of plant *Capparis decidua* were collected from village Kirri khaisore, District Dera ismail khan (kpk) Tehsil Pahar pur. The Plant *Capparis decidua* was then identified by Professor Zafarullah Zafar Department of Pure & Applied Biology Bahauddin Zakarya, Uuniversity, Multan.

Extraction

Before the process of extraction, the root barks of *Capparis decidua* were dried by placing them under the shade for 45 days, after that the root barks were grinded to obtained fine powder and then powder was weighted (2.00 kg), and with the help of successive maceration the extraction was done. The 300 gram of drug was taken and soaked in 900 milliliter of dichloromethane in closed and tight vessel in order to obtained maximum and possible extraction the vessels containing the drug was shaken after regular interval of time. After twenty four hour filter the soaked drug. After that in the eight hundred milliliter of dichloromethane, drug was again soaked. After 24 hours drug was again filter and soaked in seven hundred ml of dichloromethane. After 24 hours drug was again filtered. Then in the same manner marc was extracted by methanol (MeOH). Under the reduced pressure solvents from both methanol and dichloromethane were removed with rotatory evaporator. In order to remove both the solvents temperature of water bath was set at 30 degree centigrade. In separate wide mouth jar extracts of both dichloromethane and methanol were placed and then labeled the both extracts as CDRBD and CDRBM codes respectively. In order to complete dry both extract were placed open for one week then weighted.

Evaluation of Cytotoxic activity

The DCM extract and MeOH extract of root bark of *Capparis decidua* were checked biologically for brine- shrimp lethality assay and MTT colorimetric assay. Materials required for Brine – shrimp lethality assay were Magnifying glass, Distilled water, 2 dram vial (control + nine per sample), Sea salt (38 gram per liter of distilled water, pH 7.4), *Artemia salina* (shrimp eggs), Aluminium foil, Syringes, Test sample, Organic solvent and Pasture pipette.

Materials required for MTT colorimetric assay were 96 well plates, Minimum Essential Medium Eagle, Dulbecco's Eagle Medium, Heamocytometer, 5 % Fetal Bovine Serum, 100 uL Dimethyl sulfoxide, micro plate reader and formazan dye.

Brine – shrimp lethality assay

Generally bioactive compounds are very toxic to the *Artemia salina* shrimp larvae. In the market the eggs of brine shrimp are radially available as a fish food. The eggs hatch within the 48 hrs & produced larvae in large quantity when placed in the artificial sea water.

In this procedure dissolved twenty mill gram test sample in two milliliter of own solvent. Then 5 mL,

50 mL & 500 mL was shifted from solution to vials. The concentration sample should be 10 mcg per mL, 100 mcg per mL. The solvent was then allowed to evaporate overnight. In vials 10 larvae were put by using pasture pipette, after maturation as Nauplius and 2 days of hatching. After that up to 5mL volume was made with the sea water and then under illumination incubated at 25-28 °C for 24 hours. Those vials that were enhanced with the solvents served as a negative control while reference cytotoxic drug serving as a positive control. By using magnifying glass quantity of survived shrimps were counted and recorded. After that data was determined with the help of Finney computer program to determined LD50 values with the 95 percent confidence interval [10].

MTT colorimetric assay

To evaluate the cytotoxic potential of extract of the sample ninety six well, micro plates flat bottomed were used. In this procedure Hela cells (cervical cancer) and 3T3 cells (Mouse fibroblast) cell line was cultured in the MEME (Minimum Essential Medium Eagle) and DMEM (Dulbecco's Eagle Medium). Then in 75 cm cube flasks having 5% FBS (fetal bovine serum) that culture was supplemented. After that in five percent carbon dioxide incubator the culture was hold at thirty seven degree centigrade. Those cells which growing more rapid were harvested and these cells were counted by using the hemocytometer and while with specific medium they were diluted. Then cell culture was inoculated in

ninety six well plates. They were then incubated for whole night. After forty eight hour added 200 microliter to the wells and then incubated further for four hour. After that in each well 100 uL of Dimethyl sulfoxide was introduced. With the help of micro plate reader, absorbance was measured at the wavelength of 570 nm and extent of reduction of MTT to the formazan dye was then calculated within the cells [11]. Cytotoxicity was recorded by using the conc. of extract of sample causing fifty percent growth inhibition for HeLa cell & 3T3 cell lines. With the help of formula % inhibition was calculated [11].

$$\% \text{ inhibition} = 100 - (\text{O.d sample mean} - \text{O.d negative control mean}) / (\text{O.d positive control mean} - \text{O.d negative control mean}) \times 100$$

RESULTS AND DISCUSSION:

Brine shrimp Lethality Assay

Cytotoxic activity of both extracts i.e. MeOH and DCM of plant *Capparis decidua* was checked using brine shrimp lethality bioassay. So for this purpose both extracts such as CDRBM and CDRBD were checked. The CDRBD extract showed moderate brine shrimp larvicidal activity with LD50 value 216.1104 µg/ml, quantity of survived organism were 30 in total sum of 30 organism with the dose of 10 µg/ml, while with dose of 100 µg/ml the quantity of survived organism were 20 in total of 30 organism. While CDRBM extract showed no cytotoxic effect. Etoposide was used as standard drug in this bioassay. Result of this bioassay was shown in table 1 and 2.

Table 1: Result of bioassay of brine shrimp lethality of CDRBD samples.

Sample code	Dose(µg/ml)	Shrimps. No	Survivors. No	LD ₅₀ (µg/ml)	STD. Drug	LD ₅₀ (µg/ml)
CDRBD*	10	30	30	216.1104	Etoposide	7.4625
	100	30	20			
	1000	30	5			

**Capparis deciduas* root bark dichloromethane extract

Table 2: Result of bioassay of brine shrimp lethality of CDRBM samples.

Sample Code	Dose(µg/ml)	Shrimp No	Survive No	Std. drug	LD ₅₀ (µg/ml)
CDRBM	10	30	29	Etoposide	7.4625
	100	30	29		
	1000	30	27		

**Capparis deciduas* root bark Methanol extract

Table 3: Cytotoxic activity result of samples extracts of CDRBM and CDRBD on 3T3 cell line

Sample code	Cell lines	Con. Of sample	Con.of standard drug	% inhibition\stimulation	IC ₅₀ ± SD
CDRBD*	3T3 Cell line	30µg	Cyclohexamide(30µg)	29%	Inactive
CDRBM**	3T3 cell line	30µg	Cyclohexamide(30µg) Doxorubisin	19%	Inactive

**Capparis deciduas* root bark dichloromethane extract

***Capparis deciduas* root bark Methanol extract

Table 4: Cytotoxic activity result of samples extracts of CDRBM and CDRBD on Hela cell line

Sample code	Cell lines	Con. Of sample	Con.of standard drug	% inhibition\stimulation	IC ₅₀ ± SD
CDRBD	Hela Cell line	30µg	Doxorubisin(30µg)	30%	Inactive
CDRBM	Helacell line	30µg	Doxorubisin(30µg)	14%	Inactive

**Capparis deciduas* root bark dichloromethane extract

***Capparis deciduas* root bark Methanol extract

MTT colorimetric assay

Samples of both extracts of root bark of plant *Capparis decidua* were studied against different cell lines such as 3T3 and Hela cell lines with the concentration of 30µg/ml. Both extracts i.e. CDRBM & CDRBD of root bark of plant *Capparis decidua* were inactive against 3T3 and Hela cell line. The result also indicate that dichloromethane extract of root bark of plant *Capparis decidua* showed twenty nine percent on 3T3 cell line and 14% on Hela cell line while Methanol extract of root bark of plant *Capparis decidua* showed 19% on 3T3 cell line and 30% on Hela cell line. The results obtained are mention in table 3.& 4.

CONCLUSION:

In current study, cytotoxic activity of both extracts (DCM, MeOH) of plant through Brine shrimp lethality assay was evaluated. The DCM extract of root bark of Plant *Capparis decidua* showed moderate lethality while MeOH extract (CDRBM) showed no activity. Further research is necessary for evaluation, isolation and identification for secondary metabolites which are responsible for cytotoxic activities.

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