REPORT

Cytotoxic activities of *Rosa brunonii, Calligonum polygonoides, Pegnum harmala and Sueda fruticosa* extract using brine shrimp

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Abstract: The present study was carried out to record and evaluate the effect of *Rosa brunonii*, *Calligonum polygonoides*, *Sueda fruticosa* and *Pegnum harmala* L., extracts on brine shrimp collected during March-June 2013 from different regions of District Bannu. These four plants were medicinal xerophytes and widely distributed throughout Pakistan. *Rosa brunonii* is commonly used as a hedge plant for gardening. *Calligonum polygonoides* and *Sueda fruticosa* are locally used as a fuel, while *Pegnum harmala* (L.) is the most important multipurpose medicinal xeric plant, which is used for various purposes. All these selected medicinal xerophytes have inhibitory effect on bacterial growth. In this study the effect of different concentration (10-70 µg/ml) were tested on brine shrimp. The results showed that maximum cytotoxic activities were observed in *Rosa brunonii* (100.0±0.4), *Calligonum polygonoides* (100.0±0.2) *and Pegnum harmala* (L.) (90.0±5.2) while *Sueda fruticosa* (50.0±7.1) has less cytotoxic property. These activities are may be due to the presence of bioactive constituents.

Keyword: *Calligonum Polygonoide*, Brine shrimp, Xerophytes, Cytotoxic, District Bannu

INTRODUCTION

Plant derived drugs has been used an important remedy against various diseases in past and present (Hemalatha *et al.,* 2013, Gonzales *et al.,* 2006). Free radicals are continuously produced in the body by normal use of oxygen in respiration and also in some cell immune functions. The free radicals are also created in the body from environmental pollutants, carcinogenic agents, pesticides and certain industrial solvents causes' damage of cell membrane and other vital components in the body (Kery *et al.,* 2003). Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic and anticarcinogenic potential (Aaby and Skrede, 2004). Certain compound such as polyphenolic compounds are mostly found in both edible and inedible plants species, and they have been investigated for multipurpose biological effects such as antioxidant activity (Wojdylo *et al.,* 2007). The World Health Organization (WHO) has recognized and drawn the attention of many world countries for ever increasing interest of the public in the use of medicinal plants and their alternatives source to orthodox medicine (Baravalia *et al.,* 2011). The medicinal plants which are found in our environment enjoy wide acceptability through the population and used as cheaper antioxidant. Xeric medicinal plants contain a wide variety of antioxidant such as phytochemicals and bioactive molecule which neutralize the free radicals and retard various diseases which are associated with oxidative and reactive oxygen species (Kashani *et al.,* 2011). Antioxidants substances combat with free radicals, which cause oxidation of various compounds in living organism such as lipids, proteins and nucleic acids, which cause various diseases, such as cancer, neurodegenerative disorders, cardiovascular diseases, diabetes and other chronic diseases. The research seeks alternatives to chemicals which will reduce the dangerous effects of free radicals and improve the body's antioxidant capacity, in the form of treatment and prevention of diseases (Simao *et al.,* 2013; Joseph *et al.,* 2013). The using of plant product as an anticancer has a long history (Khalighi-Sigaroodi *et al.,* 2012). Investigations on functional plants product provide evidence on the presence of chemical which possess the beneficial ability of human health. However, there should be a necessary requirement to determine the toxic effects of some of the substances contained in the plants (Syahmi *et al.,* 2010). Investigation of plant bioactive compounds from plant extract sources in the chemical laboratory is often hampered due to absence of a suitable, simple, and rapid screening process. There are various methods are adapted for bioassay by using of whole organism, tissue or whole systems of organism. These bioassays of cytotoxicity are often complicated and expensive. A typical procedure for general toxicity screening is, therefore, necessary as a preliminary stage in plant bioactive compound study. The brine shrimp (*Artemia salina*) is used as a tool to measure general cytotoxic activity in plant extracts (Khaled and Tawaha, 2005). The aim of the present research work was to prove

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Table 1: Effect of various concentration of sample on brine shrimps

Samples	Concentration in µg/mL									
	10	20	30	40	50	60	70	LC50	DMSO	Thymol
Rosa brunonii	4.0± 0.2	5.0± 0.4	6.0± 0.4	7.0± 0.2	8.0± 0.3	9.0± 0.1	10.0 ±0.5	20.0± 0.5	0.0±0.0	10.0±0.0
Pegnum harmala	3.0± 0.3	4.0± 0.2	5.0± 0.1	6.0± 0.4	7.0± 0.2	8.0± 0.6	9.0± 0.2	30.3± 0.7	0.0±0.0	10.0±0.0
Calligonum polygonoides	4.0± 0.1	5.0± 0.3	6.0± 0.3	7.0± 0.1	8.0± 0.5	9.0± 0.2	10.0 ±0.2	20.5± 0.3	0.0±0.0	10.0±0.0
Sueda fruticosa	1.0± 0.01	2.0± 0.08	2.0± 0.08	3.0± 0.3	3.0± 0.03	4.0± 0.01	5.0± 0.05	70.7± 0.8	0.0±0.0	10.0±0.0

Table 2: Percent cytotoxicity of various samples

Sample	Concentration	%
Rosa brunonii	70 µg/ml	100.0±0.4**
Pegnum harmala	70 µg/ml	90.0±5.2**
Calligonum polygonoide	70 µg/ml	100.0±0.2**
Sueda fruticosa	70 µg/ml	50.0±7.1**
DMSO	70 µg/ml	0.0±0.0

Mean ±SD; ** shows significant P<0.05 with comparison to DMSO control

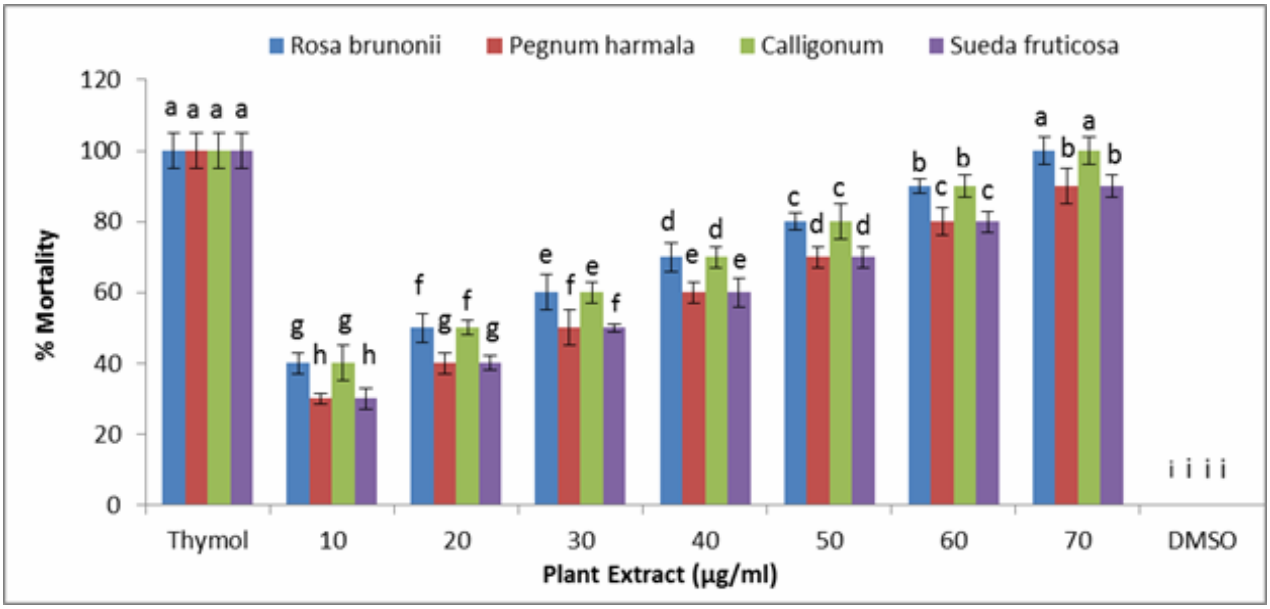

Fig. 1: Show the percent mortality of Nauplii after 24 hrs. The control were DMSO and Thymol. Error bar shows standard deviation. Letters shows significant difference.

the plant therapeutically significance of some common popular medicinal plants on the base of their cytotoxic activity.

MATERIALS AND METHODS

Leaf collection and identification

Rosa brunonii, *Calligonum polygonoide*, *Sueda fruticosa* and *Pegnum harmala* (L.) were collected from different areas of District Bannu in Khyber pakhtun Khwa, Pakistan during 2012-13. All these plants were authenticated by Prof. Yousaf and Prof. Abdur. Rehman, Department of Botany, Govt Post Graduate Collage Bannu. The leaves were washed with distilled water to remove dust and other residues and dried under shade.

Preparation of extract

400g of each plant was crushed to powder form by electric Grinder and soaked in 70% methanol for 72 hrs at room temperature. The methanol extract obtained were filtered by use of Whatman filter No.1 (Al.Zahrani and Al-Robai, 2007).

Cytotoxic assay

The Brine shrimp lethality bioassay was performed to investigate the cytotoxic (Apu *et al.,* 2010) efficacy of crude extract of *Rosa brunonii, Calligonu polygonoide, Sueda fruticosa and Pegnum harmala* (L.,). Brine shrimp (Artemia salina) eggs were placed in conical flask for 48 hrs with a continuous aeration, the eggs were hatched and active napulii were collected from conical flask. 5mg of 70% methanol extracts were exactly measured and dissolved in 1mL of Dimethyl sulfoxide (DMSO) to get a concentration of 5mg/mL. From these stock solutions, different concentrations were placed in 7 different vials making the volume up to 5mL by the NaCl solution. The final concentration of the samples extracts in the vials became 10, 20, 30, 40, 50, 60 and 70µg/mL, respectively. Serial dilutions were made in triplicate. In each vial 10 brine shrimp nauplii were placed, both positive (Thymol) and negative dimethylsulfoxide (DMSO containing sea water) control. All vials were keeping at room temperature for 24 hrs. After incubation of 24 hrs, the vials were inspected by using magnifying glass and numbers of survivor's and dead nauplii was noted and counted in each vial. The mortality ratio of this bioassay was defined as forward motion during 30 second of observation. From this observation the mortality percentage of brine shrimp nauplii for each concentration and control was calculated.

STATISTICAL ANALYSIS

A common linear (APU *et al.,* 2010) correlation was observed when logarithm of concentration versus percentage of mortality was plotted on the graph paper and the values of LC_{50} were calculated by using Microsoft Excel 2003. One way ANOVA was used for comparison with control.

RESULT

Brine shrimp bioassay was studied to determine cytotoxic activities of the four xeric medicinal plants. The result shows cytotoxicity to brine shrimp which are mention in table 1. At 70ug/ml 100.0±0.4%, 100.0±0.2% mortality were recorded in *Rosa brunonii* and *Calligonum polygonoides* but in *Pegnum harmala* (90.0±5.2)% mortily was recorded at the same amount but in *Sueda fruticosa* 50.0±7.1%. In *Rosa brunonii* and *Calligonum polygonoid* LC_{50} is obtained from graph between extract concentration and mortality (fig. 1) and found to 20µg/ml while in *Pegnum harmala* LC_{50} is obtained at 30 µg/ml and in *Sueda fruticosa* LC_{50} value obtained is 20µg/ml. The LC_{50} values obtained from brine shrimp lethality bioassay [tables 1 and 2]. LC_{50} in *Rosa brunonii*, *Pegnum harmala* and *Calligonum polygonoides* was high but in *Sueda fruticosa* cytotoxic activities are very weak. All the four medicinal xerophytes crude extract of different concentrations have different LC_{50} value in brine shrimp lethality bioassay (table 1. This significant lethality of the three plants extract indicates the presence of active potent cytotoxic components which need further investigation.

DISCUSSION

Chemical compound of plant are naturally occurring substances which work with other natural product of plants to act against diseases such as cancer or such chemical provide immunity to plants (Devasagayam *et al.*,2004). The lethal concentration for 50% mortality (LC_{50}) after 24 hrs of exposure is determined as the measure of toxicity of the extractor compound (Khaled and Tawaha. 2005). It is the simple bioassay testing the plant extracts for cytotoxic and anti-tumer activities (Krishnaraju *et al*., 2005). From different research it is clear that different plant species have good cytotoxic properties and used as a source of anticancer constituents (Ali *et al*., 2011). The research plant extract cytotoxicity on brine shrimp indicates the presence of cytotoxic constituents as well.

CONCLUSION

The cytotoxic activities indicate the presence of active compound in these plants. These plants could be of particular interest in this regard to find out its unexplored efficiency and can be a potential source for chemically interesting and biologically important drug candidates.

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