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Cytotoxic Activity of Extracts/Fractions of Various Parts of *Pistacia Integerrima* Stewart

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Abstract

The aim of present study was to scrutinize cytotoxic activity of extracts/fractions of various parts of *Pistacia integerrima* Stewart in an established *in-vitro* brine shrimp cytotoxic assay. The extracts/fractions of different parts of the plant demonstrated profound cytotoxic effect against *Artemia salina* (Leach) shrimp larvae. Of the various parts of plant tested, galls accumulated most cytotoxic agents. Among the tested extracts/fractions, hexane was least cytotoxic and therefore indicated more polar nature of cytotoxic constituents. In conclusions, extracts/fractions of various parts of *P. integerrima* exhibited marked cytotoxic profile of more polar nature.

Keywords: *Pistacia integerrim;* Extracts/fractions of different parts; Brine shrimp cytotoxic assay

Introduction

Pistacia integerrima (J. L. Stewart ex Brandis) belongs to family anacardiacea. It mostly grows at a height of 12000 to 8000 feet in Eastern Himalayan regions [1]. It is a variable sized tree that can grow up to forty feet. *P. integerrima* has been used in the treatment of inflammation, diabetes, blood purification, gastrointestinal problems, and as an expectorant. Indian are using this plant as antiasthmatic, antipyretic, antiemetic and antidiarrheal [2,3]. In Pakistan, galls of *P. integerrima* are used for treatment of hepatitis and other liver disorders [4,5]. Infections, diabetes, pain, inflammatory conditions, and fever [6].

Phytochemically, monoterpenes triterpenoids sterols, dihydromalvalic acid and flavonoids have been isolated from the different parts of *Pistacia* species [7]. In this research article, we present the results of various fractions of different parts of *P. integerrima* in *in-vitro* brine shrimp cytotoxic assay.

Material and Methods

Collection of plant materials

P. integerrima were collected from Toormang, Razagram (District Dir), Khyber Pakhtunkhwa, Pakistan in the month of February, 2010. The identification of plant material was done by Dr. Abdur Rashid plant taxonomist Department of Botany, University of Peshawar. A voucher specimen no (RF-895) was deposited in the herbarium of the same institution.

	Number of shrimps survived				
Dose	Control	Methanol	Hexane	Chloroform	Ethyl acetate
Galls					
10	0 ± 0.00	6 ± 0.00	8 ± 0.07	6 ± 0.02	7 ± 0.02
100	0 ± 0.00	3 ± 0.08	6 ± 0.02	3 ± 0.01	4 ± 0.02
1000	0 ± 0.00	0 ± 0.00	4 ± 0.00	0 ± 0.00	0 ± 0.00
Leaves					
10	0 ± 0.00	8 ± 0.02	9 ± 0.02	8 ± 0.01	7 ± 0.02
100	0 ± 0.00	7 ± 0.01	7 ± 0.01	7 ± 0.02	5 ± 0.01
1000	0 ± 0.00	5 ± 0.02	4 ± 0.01	5 ± 0.00	4 ± 0.01
Bark					
10	0 ± 0.00	7 ± 0.02	8 ± 0.02	8 ± 0.00	7 ± 0.02
100	0 ± 0.00	4 ± 0.01	6 ± 0.01	6 ± 0.01	3 ± 0.00
1000	0 ± 0.00	0 ± 0.00	4 ± 0.00	4 ± 0.00	0 ± 0.00
Roots					
10	0 ± 0.00	7 ± 0.02	10 ± 0.00	6 ± 0.01	8 ± 0.00
100	0 ± 0.00	5 ± 0.01	9 ± 0.02	3 ± 0.00	4 ± 0.01
1000	0 ± 0.00	2 ± 0.01	7 ± 0.00	0 ± 0.00	2 ± 0.01

Table 1: Cytotoxic effect of extracts/fractions of different parts of *P. integerrima* at 10,100 and 1000 µg/ml. Data are three mean ± SEM of three different experiments.

Extraction and fractionation

Shade dried and crushed galls, leaves, barks and roots of *P. integerrima* (Stewart) were macerated with methanol at room temperature for one week. The resulting methanolic residue of each part was evaporated at 40°C using rotary evaporator. For fractionation, each crude solvent was suspended in water and sequentially fractionated with hexane, chloroform and ethyl acetate in order to get their respective fractions. The commercial solvents, after distillation were used for extraction and fractionation.

Brine shrimp cytotoxic assay

In-vitro brine shrimp cytotoxic assay was used for the assessment of extracts/fractions of different parts of the plant [8]. Briefly, test samples were prepared in respective solvents in the concentrations of 10, 100, and 1000 µg/mL. Brine shrimp (*Artemia salina* Leach) nauplii were hatched in a specific tank at room temperature. Form stock solutions, 5, 50 and 500 µg/mL were injected into 9 vials (3 vials for each dilution). Each vial contained ten shrimps and 5 ml of brine. The vials were supplemented with dry yeast suspension as their food and were incubated for 24 h under illumination. For analysis, the live nauplii were counted with the aid of a 3 x magnifying glass and thus calculated the percent deaths at each dose. The resulting data were processed by using GraphPad Prism version 6. LD_{50} values were the mean of three replicates.

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Data analysis

The resulting data is presented as mean of three independent assays. The statistical software, GraphPad Prism version 6 used was for the estimation of LD_{50}.

Results

The effects of cytotoxic activity of crude extracts and various solvent fractions of different parts of *P. integerrima* are illustrated in Table 1.

Cytotoxic effect of galls of *P. integerrima*

The results of galls of extract/fractions of *P. integerrima* against *A. salina* are shown in Figure 1. The extract/fractions exhibited significant cytotoxic effect at various test concentrations of 10, 100 and 1000 µg/ml.

Cytotoxic effect of leaves of *P. integerrima*

The effects of cytotoxic assay of leaves of *P. integerrima* are illustrated in Figure 2. The results showed marked inhibition on the growth of *A. salina* when tested at various concentrations (10, 100 and 1000 µg/ml).

Cytotoxic effect of barks of *P. integerrima*

Regarding cytotoxic effect of crude extract and subsequent solvent fractions of barks of the plant, marked mortality was observed against *A. salina* at test concentrations of 10, 100 and 1000 µg/ml (Figure 3).

Cytotoxic effect of roots of *P. integerrima*

As shown in Figure 4, similar patron of cytotoxicity was demonstrated by the crude extract and subsequent solvent fractions of roots of the plant. The overall effect was in a concentration dependent manner.

Discussion

Brine shrimps cytotoxic assay is a simple, quick and economical bioassay which has been specially designed to evaluate the cytotoxic potential of active natural products; both extracts and pure isolated compounds. The eggs of the brine shrimp, *A. salina* are readily available as fish food in pet shops. When placed in artificial seawater, the eggs hatch within 48 h, providing large numbers of larvae [9,10]. Experimental findings have shown that *A. salina* (Leach) shrimp larvae exhibited profound sensitivity to bioactive compounds [11].

The results of our study showed that the extracts/fractions of various parts of *P. integerrima* had marked cytotoxic effects on brine shrimp larvae. When tested at various concentrations (10, 100 and 1000 µg/ml), among the different parts of the plant, extracts/fractions of galls were observed most cytotoxic. The results indicating that the cytotoxic constituents of the plant are concentrated in galls of the plant. Upon fractionation, the *n*-hexane was the less sensitive fraction towards *A. salina* larvae. It suggested that the cytotoxic constituents of the plant in principal are more polar in nature. However, the isolation of pure secondary metabolites from the different parts of the plant could provide us better understanding of the phytochemical profile of bioactive components.

In the discovery of new potential anticancer drugs, natural cytotoxic agents have been considered to be a valuable pool of lead compounds.

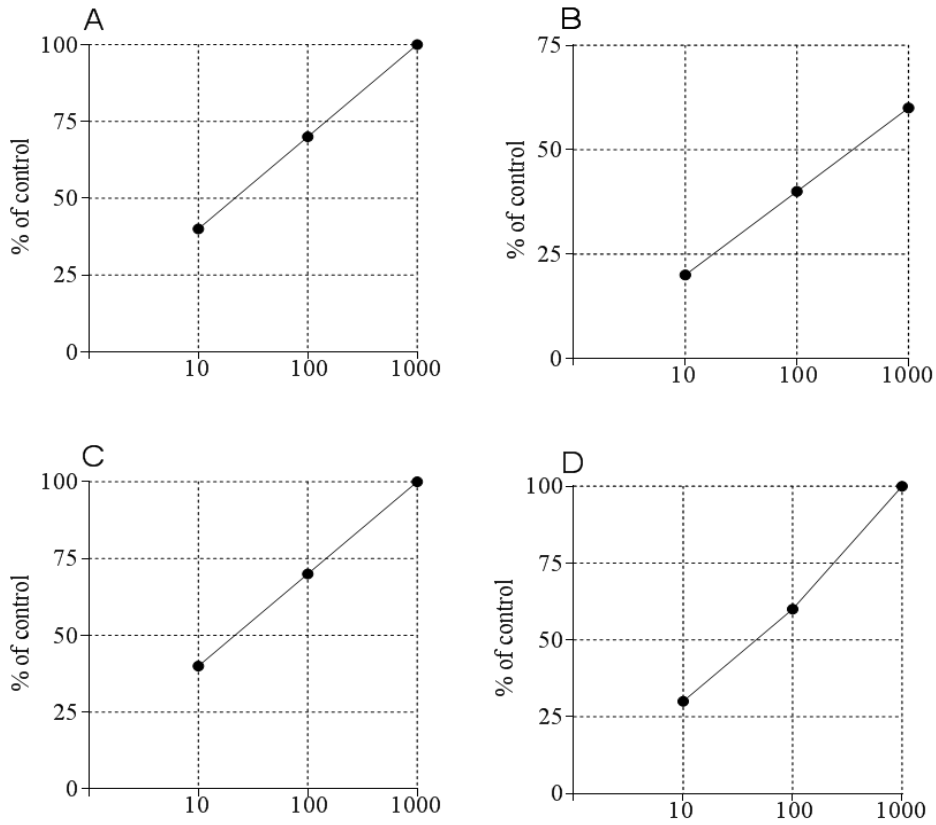

Figure 1: Effect (%) of extracts and fraction of galls of *P. integerrima against A. salina* larvae at various concentrations. [A] Crude extract, [B] hexane fraction, [C] chloroform fraction, [D] ethyl acetate fraction. Data are the results of mean of three independent assays.

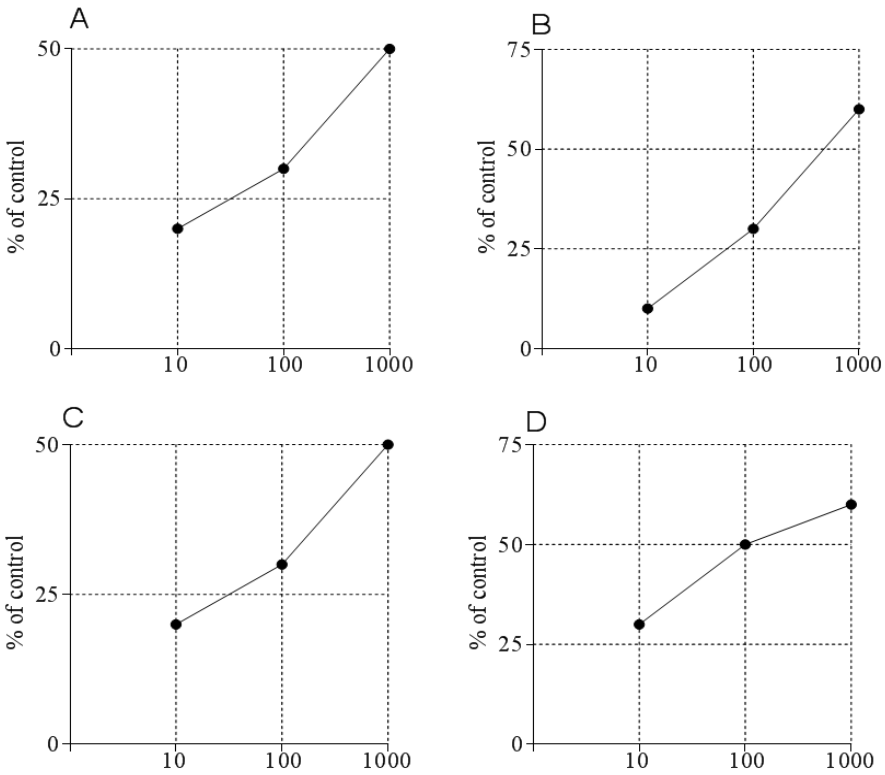

Figure 2: Effect (%) of extracts and fraction of leaves of *P. integerrima* against *A. salina* larvae at various concentrations. [A] Crude extract, [B] hexane fraction, [C] chloroform fraction, [D] ethyl acetate fraction. Data are the results of mean of three independent assays.

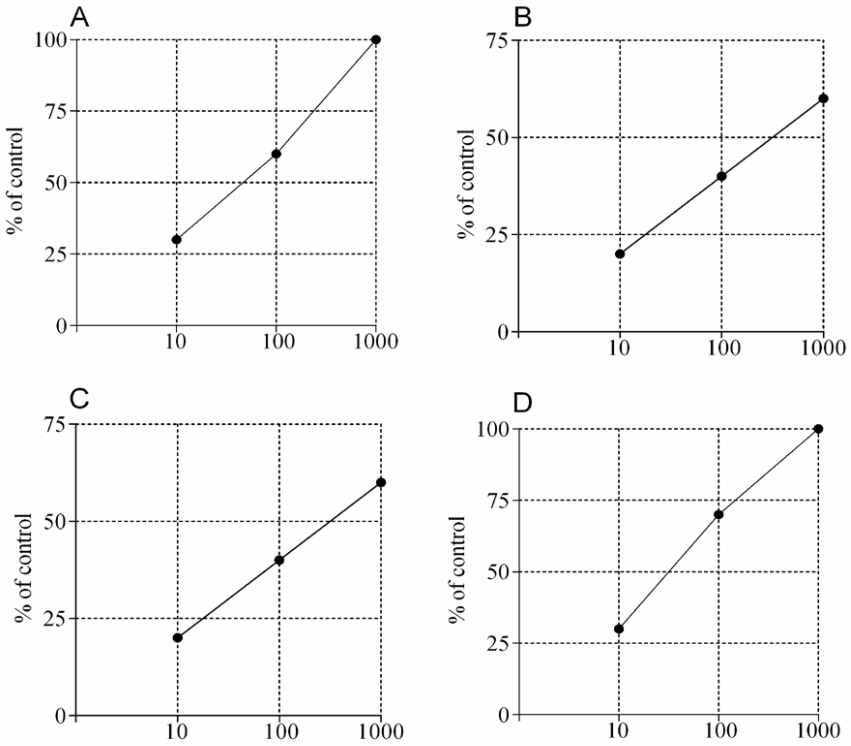

Figure 3: Effect (%) of extracts and fraction of barks of *P. integerrima against A. salina* larvae at various concentrations. [A] Crude extract, [B] hexane fraction, [C] chloroform fraction, [D] ethyl acetate fraction. Data are the results of mean of three independent assays.

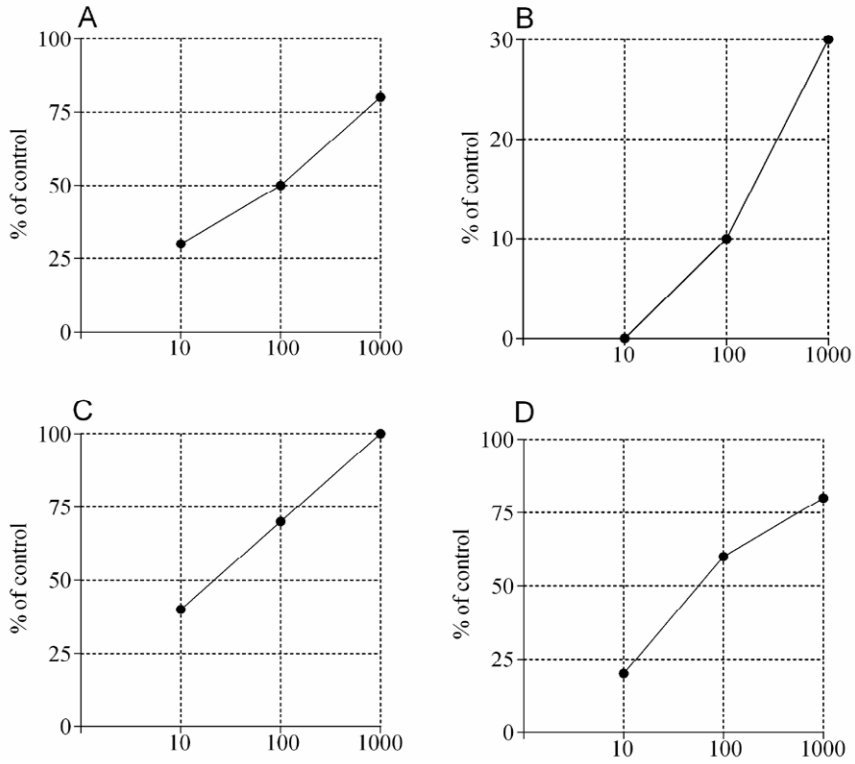

Figure 4: Effect (%) of extracts and fraction of roots of *P. integerrima against A. salina* larvae at various concentrations. [A] Crude extract, [B] hexane fraction, [C] chloroform fraction, [D] ethyl acetate fraction. Data are the results of mean of three independent assays

In current clinical practice, some cytotoxic compounds of terrestrial plants are being used as cancer therapeutic drugs and more candidates are under clinical trials along with cytotoxic small molecules of terrestrial microorganisms and marine organisms [12]. The researchers also pointed that some of the plant based isolated compounds/extracts had cytotoxic action other than the standard drugs available for the treatment of different cancers and thus suggested some different mechanism(s) for their cytotoxicity [13].

It is concluded on the basis of results that the extracts/fractions of *P. integerrima* possess strong cytotoxic constituents and could be a useful new source as cytotoxic agents. While considering this study as a foundation, further details are most warrant in terms of isolation of pure molecules in order to justify the phytochemical background and the mechanism(s) for the cytotoxic effect.

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