In Vitro Antimicrobial, Antioxidant and Cytotoxic activities of *Polygonum orientale* (Bishkatali)

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Abstract: The aim of this present work is to investigate the antimicrobial, antioxidant and cytotoxic activities of methanol extract of *Polygonum orientale* (Family: Polygonaceae). Antimicrobial activity of methanol extract of *Polygonum orientale* was tested by Disc Diffusion Method. Standard antibiotic discs of Kanamycin (30µg/disc) for bacterial species was used as standard and crude extracts were used at a concentration of (20mg/disc) and (40mg/disc) for leaves and (10mg/disc) and (20mg/disc) for shoot extracts. The methanol extracts of leaves and shoots showed mild activity against gram positive bacteria, gram negative bacteria and fungi. Most of the organisms were susceptible to the extract in various degrees. The extract showed highest zone of inhibition (13mm) against the Gram (+)ve *Bacillus megaterium*and and gram (-)ve *Escherichia coli* (zone of inhibition 10mm). Antioxidant property of the plant extracts against stable DPPH (1, 1-Dipheny1-2-picrydrazole) radical was investigated. Ascorbic acid was used as standard. The extract was found low in antioxidant property. The absorbance was taken at 517nm by UV Spectrophotometer. The IC_{50} values were found in leaf extract and shoot extract are (1244.58µg/ml and (1506µg/ml). For ascorbic acid IC_{50} value was (139.19µg/ml). Cytotoxic activity of the crude extract by using Brine Shrimp Lethality Bioassay. Vincristin sulphate was used as standard. LC_{50} value of leaf extract was found (6.85µg/ml) and (9.7104µg/ml) for the shoot extract. In conclusion, the extract of the experimental plants have mild antimicrobial, low antioxidant and good cytotoxic properties.

Keywords: *In vitro*, Antimicrobial, Cytotoxic, DPPH scavenging, Polygonaceae.

INTRODUCTION

Medicinal plants are considered as rich resources of ingredients which can be used in drug development and drug synthesis. Besides that these medicinal plants play a critical role in the development of human cultures about plants around the whole world [1]. The plant kingdom continues to be a foremost source of novel natural products with potential for use as drugs or pharmaceutical mediators. According to The World Health Organization, more than 80% of the world population in developing countries depends primarily on plants based medicines for basic healthcare needs [2]. Medicinal plants serve as important therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and modern medicines. They offer alternative remedies with tremendous opportunities to generate income, employment and foreign exchange for developing countries [3]. It has further been observed that a number of modern pharmaceuticals have been derived from plants used by indigenous people. Important modern drugs that have been derived from observations of traditional curing methods of indigenous people include aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine [4]. *Polygonum orientale L.* (Family: Polygonaceae) is a tall annual, with fistulous branches, 0.9-3 m high, stems robust and grooved. Leaves of *Polygonum orientale L.* is 15-23 cm long, ovate or ovate cordate, long-pointed, stipules short, truncate, hairy, ciliate at the mouth. Flowers of *Polygonum orientale L.* is white or red in dense, erect or drooping racemes, 5-10 cm long. It is commonly known as Bara Panimarich, Bishkatali (Bengali). The plants have a good tonic and vulnerary used for healing wounds .The plant contains β - sitosterol, orientoside and orientin. It is distributed throughout the country in damp, lowlying areas, canal banks [5].

MATERIALS & METHODS

Preparation of Plant Materials

The plant materials were collected in fresh condition and then these are cut into small pieces and dried in the ambient temperature. Then the dried pieces are ground into coarse powder with the help of a grinder

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and stored in airtight containers for further use. The plant material, after drying at ambient temperature and milling, was individually extracted by maceration with methanol. Crude extract was obtained after concentrating the solution on a rotary evaporated and drying at surrounding temperature. The process of removing organic compound from its aqueous solution by shaking with a suitable organic solvent is termed as solvent-solvent partitioning. It is always better to extract two or three times with smaller quantities of the solvent than once with the bulk of the solvent provide. This is done in a separating funnel [6].

Chemical Investigation of *Polygonum orientale*

The fresh leaves and shoots of the plant *Polygonum orientale* was collected during the month of May to June (2014) from the area of Munshigonj in Bangladesh. The plant *Polygonum orientale* was taxonomically identified by The National Herbarium. The plant powder materials leaves (158gm) and shoots (100gm) were extracted by cold extraction process with methanol (700ml) and (400ml) in a flat bottom glass container respectively. Through occasional shaking and stirring for 15 days. The extract was then filtered through cotton and filter paper. The filtrates were concentrated to afford solid masses by using a water bath at 40ºC [7].

In Vitro Antimicrobial Screening

The antimicrobial screening was performed by the disk diffusion technique [8, 9]. In our present study, the antimicrobial activity of methanol extract of plant was investigated in comparison with standard Kanamycin (30µg/disc) antibiotic against a number of pathogenic gram-positive and gram-negative bacteria and fungi. Both gram-positive and gram-negative strains of bacteria and fungus were used as the test organisms to observe the anti-microbial and anti fungal activity of the compounds. These organisms were collected from the microbiology research laboratory, Department of Pharmacy, Southeast University. The pure culture of these organisms was previously collected from the Microbiology Department of Dhaka University. Dried methanol extracts of *Polygonum orientale* plant leaves (20 mg) and (40mg) in each were dissolved in methanol (10ml in each) to get two concentrations in each (200 mg/10ml) and (400mg/10ml) respectively. Dried methanol extracts of *Polygonum orientale* plant shoots (10 mg) and (20mg) in each were dissolved in methanol (10ml in each) to get two concentrations in each (100 mg/10ml) and (200mg/10ml) respectively. The sample disc and standard antibiotic discs were placed gently on the solidified agar plates freshly seeded with the organism with the help of a sterile forceps to ensure complete contact with medium surface. The arrangement of the disc was such that the discs were no closer than 15 mm to the plate to prevent overlapping of the zone of inhibition. The plates were then inverted and kept in a refrigerator for about 24 hours at 4ºC. This was sufficient time for the material to diffuse to a considerable area of the medium. Finally, the plates were incubated at 37ºC for 12-18 hours.

Antioxidant Activity

The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams. Methanolic extract of *Polygonum orientale* was mixed with 95% methanol to prepare the stock solution (500mg/10ml).The concentration of this solution is 5mg/100ml or 5000µg/ml. Then 4ml of methanol extract of the extract at different concentrations (500, 250, 100, 50, 25µg/ml)

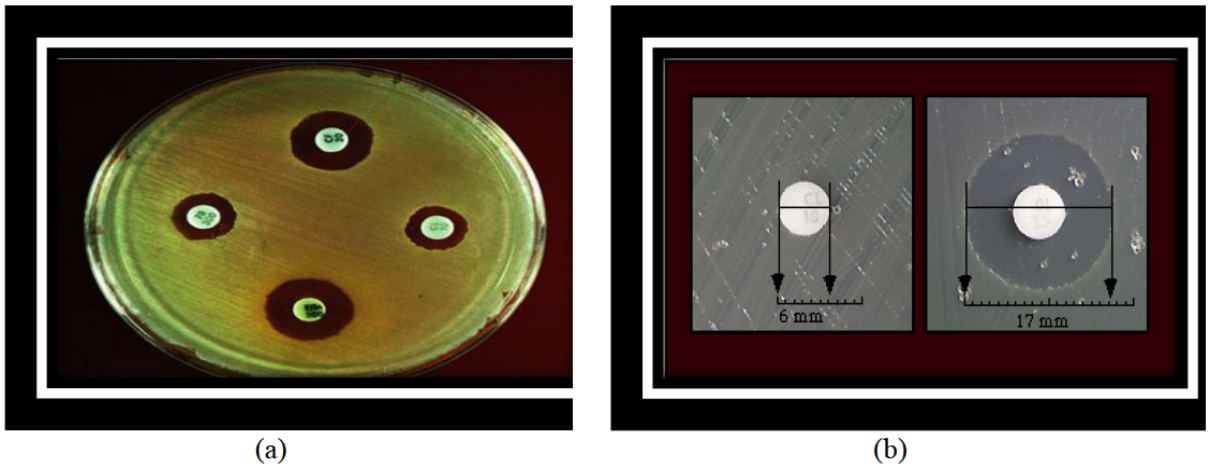

Figure 1: a) Clear zone of inhibition (**b**) Determination of clear zone of inhibition.

were mixed with 100ml of DPPH methanol solution (200µg/ml). After 30mins of reaction period at room temperature in dark place the absorbance was measured against 517nm methanol as blank by UV spectroscopy .Inhibition of free radical DPPH in percentage (I%) was calculated as follows:% DPPH radical scavenging = [(Absorbance of control – Absorbance of test sample)/ (Absorbance of control)] X 100 or (I) % = (1- A_{sample}/A_{blank}) X 100 [10-12].

Determination of Brine Shrimp Lethality Bioassay (Cytotoxicity Test) [13-16]

Artemiasalina leaches (Brine Shrimp) were collected from pet shops which were used as test organism. Seawater (38gm) was taken in the small tank & shrimp eggs were added to one side of the tank & then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through time .The hatched shrimps were attracted to the lamp through the perforated dam & they were taken for experiment.The methanolic extracts of leaves and shoots were taken for the operation. Then 4 mg of extract was dissolved 5ml of dimethylsulfoxid (DMSO) to get the concentration of 1µg/1µl. From each test tube of these test solutions 100µl were added to premarked glass vials/test tubes containing 5ml sea water and 10 shrimp nauplii. So the final concentration of samples in the vials/test tubes were 400µg/ml, 200µg/ml, 100µg/ml, 50 µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml, 0.78125µg/ml respectively [17, 18].

Preparation of the Vincristine Sulphate Test Solution

Vincristine sulphat served as the positive control. 2 mg of vincristine sulphate was dissolved in DMSO to get an initial concentration 20 µg/ml from which serial dilution were made using pure dimethyl sulfoxide DMSO to get 10µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625µg/ml, 0.312µg/ml, 0.15625µg/ml, 0.078125 µg/ml, 0.0390µg/ml respectively. After 24 hours, the vails were observed; the number of survived nauplii in each vial was counted & noted. From this data, the percentage of lethality of the nauplii was calculated at each concentrate [19].

RESULTS

Antimicrobial Activity of *Polygonum orientale*

The antimicrobial activity of *Polygonum orientale* was shown in Tables **1**, **2** and **3**.

The extracts showed low to moderate antibacterial activity against most of the pathogenic bacteria. This extract showed moderate antimicrobial activity against the Gram-(+) *Bacillus megaterium* (13mm) and *Sarcina lutea* (12mm) and the Gram-(-) *E. coli* (10 mm) bacteria for leaf extract in the concentration of (40mg/disc) with an average zone of inhibition of (6 – 13) mm. *Shigella boydii* and *Pseudomonas aeruginosa* have shown no antimicrobial activity against all the concentrations.

Result of the Antioxidant Activity

Antioxidant property of the extract was compared with ascorbic acid by its DPPH free radical scavenging activity of *Polygonum orientale* are shown in Table **4**. At a concentration of (500µg/ml) metanolic extract of *Polygonum orientale* scavenged 32% DPPH free radical.

Different percentage of extracts of Polygonum orientale was subjected to free radical scavenging activity by DPPH method. In this investigation, the

Table 1: Leaf Extracts for Organisms
The zone of inhibition produced by the extracts at a concentration of (20mg/disc) and (40mg/disc) for leaf extracts. The diameters of zone of inhibitions against tested microorganisms are shown in table.

Leaf extracts used in Gram positive bacteria	Concentration (20mg/disc)	Concentration (40mg/disc)	Standard (Kanamycin Concentration 30µg/disc)	Leaf extracts used in Gram negative bacteria	Concentration (20mg/disc)	Concentration (40mg/disc)	Standard Kanamycin Concentration (30µg/disc)
Bacillus subtilis	6	7	20	*Pseudomonas aereuginosa*	0	0	18
Bacillus megaterium	3	13	25	*Vibrio mimicus*	8	8.5	7
Staphylococcus aureus	6	7	30	*Shigella boydii*	0	0	12
Sarcina lutea	10	12	34	*E. coli*	9	10	30
Bacillus cereus	6	9	23	*Vibrio parahaemolyticus*	6	6.5	22

Table 2: Shoot Extracts for Organisms

The zone of inhibition produced by the extracts, at concentrations of 10mg/disc and 20mg/disc for leaf extracts. The diameters of zone of inhibitions against tested microorganisms are shown in table.

Shoot extracts used in Gram positive bacteria	Concentration (10mg/disc)	Concentration (20mg/disc)	Standard Kanamycin Concentration (30µg/disc)	Shoot extracts used in Gram negative bacteria	Concentration (20mg/disc)	Concentration (40mg/disc)	Standard Kanamycin Concentration (30µg/disc)
Bacillus subtilis	0	0	20	*Pseudomonas aereuginosa*	0	0	18
Bacillus megaterium	0	0	25	*Vibrio mimicus*	2	2	7
Staphylococcus aureus	6	6	30	*E. coli*	7	8	30
Sarcina lutea	6	7	34	*Vibrio parahaemolyticus*	6	6	23
Bacillus cereus	6	6.5	23	*Shigella boydii*	0	0	12

Table 3: Leaf and Shoot Extracts Used in Fungus

Leaf extracts used in Fungus	Concentration (10mg/disc)	Concentration (20mg/disc)	Standard Kanamycin Concentration (30µg/disc)	*Shoot extracts used in* Fungus	Concentration (20mg/disc)	Concentration (40mg/disc)	Standard Kanamycin Concentration (30µg/disc)
Aspergillus niger	6	7	17	5	6	17	5
Sacharomyces cerevaceae	7	8	22	0	6	22	0

Table 4: DPPH Radical Scavenging Activity of Polygonum Orientale Extracts and Ascorbic Acid

Concentration (µg/ml)	Absorbance of blank+DPPH(200 µg/ml)	Absorbance of leaf extract	Absorbance of shoot extract	Absorbance of ascorbic acid	% inhibition of leaf extract	% inhibition of shoot extract	% inhibition of ascorbic acid	IC_{50} value of leaf extract	IC_{50} value of shoot extract	IC_{50} value of ascorbic acid
25	3.67	2.482	3.311	3.097	19	9	15	1244.58	1506	139.19
50	3.67	2.798	3.215	2.553	21	12	30			
100	3.67	2.872	3.156	1.830	21	14	50			
250	3.67	2.873	3.046	0.087	23	17	97			
500	3.67	2.960	2.825	0.085	32	23	97			

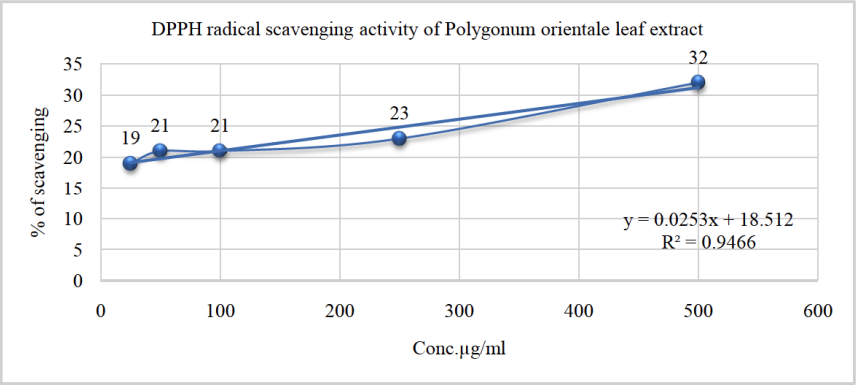

Figure 2: DPP*H* radical scavenging activity of *Polygonum orientale* leaf extract

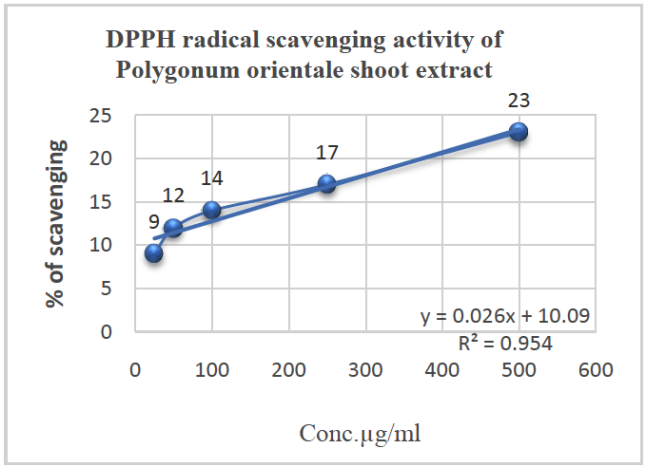

Figure 3: DPPH radical scavenging activity of *Polygonum orientale* Shoot extract.

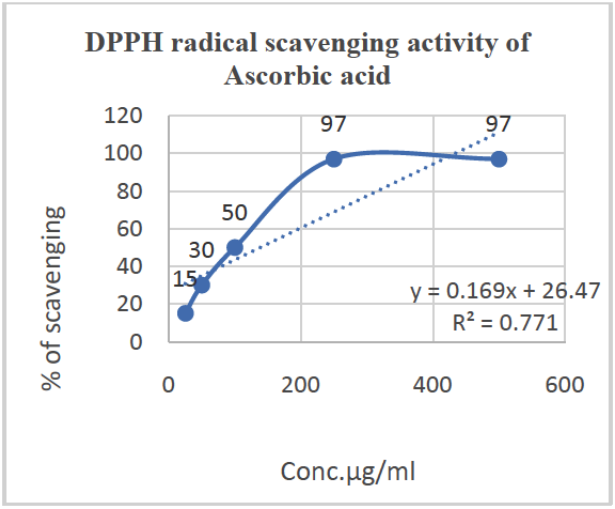

Figure 4: DPPH radical scavenging activity of Ascorbic acid.

extracts of methanol showed the activity with IC_{50} value accordingly (1244.58µg/ml) for leaf extract and 1506µg/ml for shoot extract. The IC_{50} value of the extract was (1244.58µg/ml) and (1506µg/ml) as opposed to that of ascorbic acid IC_{50} (139.19µg /ml). Thus demonstrating low antioxidant activity.

Cytotoxicity Activity of *Polygonum orientale*

Brine Shrimp Lethality Bioassay

In the present bioactivity study all the crude extract showed positive results that the test samples are

Table 5: Effect of Vincristine Sulphate on Brine Shrimp Nauplii

Concentration(C) (µg/ml)	Log C	% of Mortality (Vincristine Sulphate)	LC_{50}(µg/ml)
20	1.30103	100	0.73
10	1	90	
5	0.69897	90	
2.5	0.39794	80	
1.25	0.09691	70	
0.625	-0.20142	60	
0.3125	-0.50515	40	
0.15625	-0.80616	30	
0.078125	-1.10721	30	
0.0390	-1.40894	20	

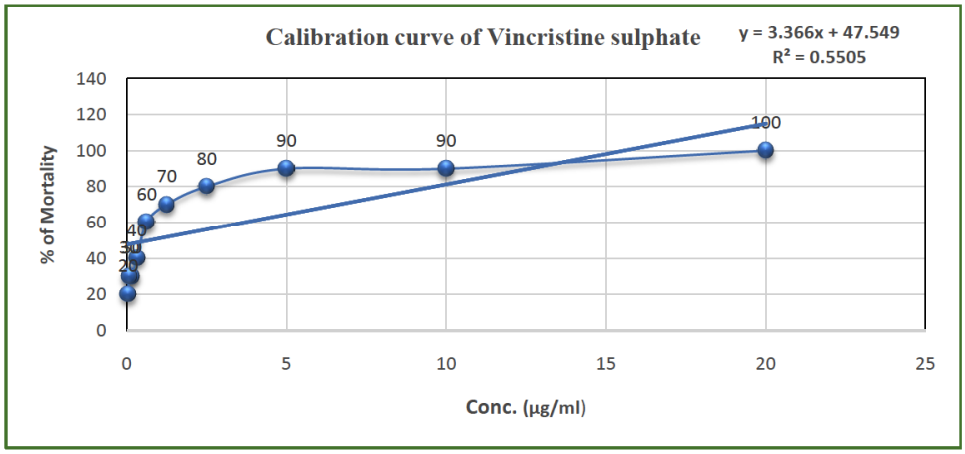

Figure 5: Calibration Curve of Vincristine sulphate.

Table 6: Investigation of Cytotoxicity Test of Polygonum orientale Leaf Extract

Concentration	Log of concentration	No. of nauplii added	No of nauplii alive	No. of nauplii dead	%of mortality	LC_{50}(µg/ml)
400	2.602	20	0	20	100	6.85
200	2.301	20	0	20	100	
100	2.000	20	4	16	95	
50	1.699	20	17	14	85	
25	1.398	20	17	13	75	
12.5	1.097	20	8	12	75	
6.25	0.796	20	10	10	75	
3.125	0.495	20	11	9	65	
1.562	0.194	20	13	7	45	
0.781	-0.107	20	17	3	70	

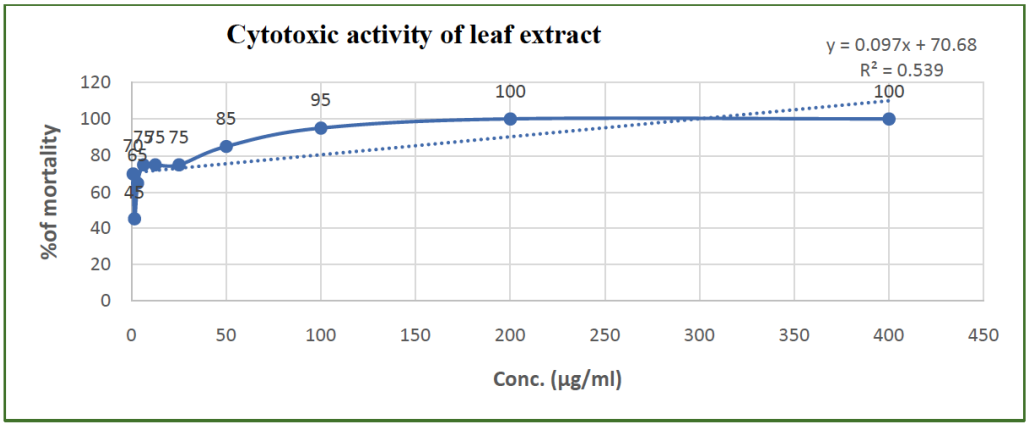

Figure 6: Effect of *Polygonum orientale* leaf extract on brine shrimp.

Table 7: Investigation of Cytotoxicity Test of *Polygonum orientale* Shoot Extract

Concentration (C)	Log of concentration (C)	No. of nauplii added	No of nauplii alive	No. of nauplii dead	%of mortality	LC_{50}(µg/ml)
400	2.602	20	0	20	100	9.7104
200	2.301	20	0	20	100	
100	2.000	20	2	18	90	
50	1.699	20	3	17	85	
25	1.398	20	3	17	85	
12.50	1.097	20	6	17	85	
6.25	0.796	20	6	14	70	
3.125	0.495	20	6	14	70	
1.562	0.194	20	7	13	65	
0.781	-0.107	20	11	9	45	

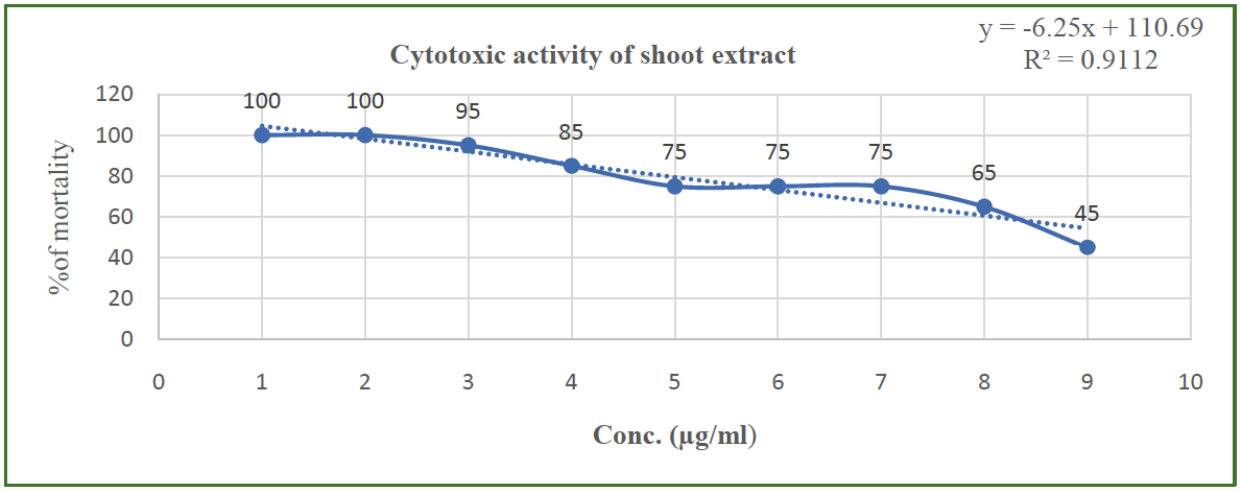

Figure 7: Investigation of Cytotoxicity Test of Polygonum orientale Shoot Extract.

LC_{50} value of 0.73µg/ml was found for Vincristine sulphate and the results have found for the leaf extract LC_{50} 6.85µg/ml and for the shoot extract LC_{50} 9.7104µg/ml. The extracts showed good cytotoxic activity.

biologically active. Each of the test samples showed different mortality rates at different concentration [20].

CONCLUSION

In conclusion, the present study demonstrates that the plant, *Polygonum orientale* have mild antmicrobial, low anti-oxidant and good cytotoxic activities. Therefore, the plant may be utilized for the development of traditional medicine and further investigation is necessary for the development of the lead compound and to establish it as a safer anticancer agent.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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